EFFECTS OF SELECTION FOR MILK YIELD ON DAIRY CATTLE PERFORMANCE AND ENDOCRINE REGULATION

by

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ABSTRACT

The effects of selection on body weight (BW), dry matter intake (DMI), milk yield, fat percent, and on plasma concentration of insulin (INS), growth hormone (GH), and Insulin-like Growth Factor I (IGF-I) were studied in two groups of first lactation Holstein cows of differing genetic merit (selection vs control). Dry matter intake (DMI) was measured at 45, 90, 180, 225, 270, and 315 d postpartum. Serial blood samples were collected at 30 d intervals for a 5 hr period at 15 min intervals. Selection group cows were heavier (532 vs 514 kg, N.S.) than control cows with a group by days in milk (DIM) interaction ($P<.01$). Significant differences ($P<.05$) in energy intake occurred with an average of 25.6 Mcal/d for the control group and 28.6 Mcal/d for the selection group cows. Milk yield and mean milk fat were greater ($P<.01$) in the selection group cows. The mean estimated production efficiency (kg milk/Mcal intake) was .84 and 1.02 ($P<.05$) for the control and selection group, respectively. Plasma GH was higher ($P<.01$) and IGF-I was lower ($P<.1$) in selection group cows compared to control cows. Mean plasma INS concentrations were 821 vs 763 pg/ml (N.S.) for control and selection group cows. A significant ($P<.01$) interaction occurred between group and month of lactation for GH. The mean IGF-I plasma levels were 170 ng/ml and 139 ng/ml ($P<.1$) for the control versus the selection cows respectively. The results indicate that selection for milk yield resulted in

differences in DMI, milk fat and plasma concentrations of GH and IGF-I. Selection also resulted in increased estimates of production efficiency.

In a follow up study the effects of selection on BW, DMI, milk yield, fat percent and response to Growth Hormone Releasing Factor (GRF) as well as glucose infusion were studied in early lactation control and selection group cows. Dry matter intake was measured at 45 and 90 DPP. Serial blood samples were collected at 15 d intervals for a period of either 6 or 15 hr at various intervals. Selection group cows were similar in body weight (494 vs 489, N.S.) than control cows, however energy intake tended to be greater for selection group cows than for control animals (23.3 vs 20.4 Mcal/d, $P<.1$). Milk yield was greater in selection group cows ($P<.01$). The mean estimated production efficiency was 1.31 vs 1.18 (kg milk/Mcal intake) (N.S.) for the selection and control group, respectively. Mean plasma GH was higher on all test days (30, 60, 90, 120 DPP) for selection compared to control group cows (15.6 vs 23.5 ng/ml, $P<.01$). Mean plasma INS concentrations were 1017 vs 1032 pg/ml (N.S.) for the control and selection groups, respectively, following glucose infusion(.1mg/kg BW). Mean plasma IGF-I concentrations tended to be greater ($P<.14$) in control group cows compared to selection group cows. No increase in plasma IGF-I was observed in the thirteen hours following GRF (.2ug/kg BW) administration in either group of cows. The results indicate that selection for milk yield resulted in difference in DMI and plasma concentration of GH in response to GRF infusion but it did not affect plasma INS.

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LITERATURE REVIEW

The endocrine system plays an essential role in the regulation of mammary development and function (Tucker, 1981; Massri et al., 1985; Sejrsen and Lovendahl, 1986). Two obvious candidates as predictors of genetic merit for milk yield and increased production efficiency are mammary active hormones and regulators of the secretion of such hormones. The estimation of the heritability of hormone concentration and relationship between the hormone and measurable production traits could lead to the identification and selection of young animals with superior milk yield potential.

Bauman et al. (1985) reviewed the possibility that physiological traits may be used as indicators of genetic merit for milk yield and concluded that first, the physiological factors that contribute to variation in production efficiency must be identified and secondly, the extent too which these factors are sensitive to genetic manipulation must be determined. Both growth hormone (GH) and insulin (INS) have been investigated as candidates which may be altered by genetic selection because of their direct or indirect involvement in the initiation or maintenance of lactation (Hart et al., 1978; Falconer et al., 1980).

Two distinct types of regulation are involved in partitioning nutrients to various body tissues. Homeostasis is an acute minute by minute control, and homeorhesis is an orchestrated change in metabolism in the directed partitioning

of nutrients to support differing physiological states, for example, lactation or pregnancy (Bauman and Currie, 1980). A homeorhetic example would be the initiation of lactation in the dairy cow, a process which dramatically alters the metabolism of many maternal organs to supply the mammary gland with the nutrients necessary for milk synthesis. Milk synthesis and secretion proceeds at the expense of other metabolic processes (Hickman, 1980). The nutrient needs of the mammary gland are of such magnitude relative to the total metabolism in high producing cows, that, for practical purposes, the cow may be considered an appendage on the udder rather than the reverse (Brown, 1969). Alterations in metabolism which partition nutrients to the mammary gland are key to the initiation and maintenance of a successful lactation.

Several studies have suggested that digestion, nutrient absorption, maintenance requirement and utilization of metabolic energy for milk production vary little between animals and remain relatively unaffected by selection for increased milk yield, but individual cows may vary considerably in the way in which they partition absorbed nutrients (Swan, 1976; Bauman and Currie, 1980; Moe, 1981; Hart, 1983). Bines and Hart (1978) suggest that genetic make-up is responsible for the origination of differences in nutrient partitioning, which is further mediated by differences in endocrine balances. One of the best known physiological responses to selection for milk yield, as reported by Hickman (1980), is a complex genetic mechanism which maximizes the amount of and availability

of mobilizable adipose tissue at freshening. High-yielding cows divert dietary energy and mobilize body tissues to attempt to meet the high demands of the lactating udder during early and mid-lactation (Hart et al., 1979). Concomitantly, low-yielding cows consuming the same ration, partition a varying proportion of nutrients to anabolic processes which increase body weight (Hart et al., 1979).

During early lactation nutrient requirements greatly exceed the nutrient intake in high-yielding dairy cattle (Bauman et al., 1980). Metabolic processes such as lipid metabolism in adipose tissue and gluconeogenesis in the liver must be coordinated to provide sufficient nutrients for milk synthesis. Since blood levels of GH are higher and INS levels lower early in lactation (Koprowski et al., 1973; Smithe et al., 1975; Vasilatos et al., 1981) it's thought that the ratio of GH to INS in plasma may be a key regulator in the partitioning of absorbed nurtrients for milk production (Bines et al., 1980). Certainly, GH increases the rates of lipolysis in several species (Tata, 1976) and the antilipolytic role of INS is well established (Newsholme et al., 1973).

Dairy cows fed the same quantities of a diet can differ markedly in milk yield. These differences may be primarily attributed to nutrient partitioning between body stores and milk synthesis (Bauman et al., 1980). Plasma hormone concentrations, especially GH and INS, appear to play an important role in nutrient partitioning (Bines et al., 1982). High-yielding dairy cows have higher plasma GH concentrations than do low producing cows on similar dietary regimen

(Bines et al., 1982). Although differences in energy balance may contribute to differences observed in plasma hormone concentrations, GH and INS concentrations in plasma are correlated to milk yield (Hart et al., 1980). Injections of bovine somatotropin (bST) increase milk yield (De Boer et al., 1989), whereas milk yield declines in response to INS injections (Schmidt, 1968). In general, higher plasma concentrations of GH promote milk yield, whereas higher plasma concentrations of INS promote deposition of nutrients in peripheral tissue such as adipose tissue. Maintaining a high GH:INS ratio is important in maximizing milk yield (Bines et al., 1982). Somatotropin decreases nutrient uptake by peripheral tissue and increases mobilization of energy from peripheral tissues, especially adipose tissue. Insulin appears to have the opposite effect (Bines et al., 1982). Therefore, simultaneously increasing plasma GH and decreasing plasma INS additively affect milk yield.

Research has shown circulating GH concentration to be related to certain metabolic events associated with nutrient partitioning (Bines and Hart, 1978; Bauman et al., 1985). One example is lipolysis, the mobilization of fat tissue stores in times of energy deficit. Growth hormone inhibits proteolysis and stimulates the incorporation of amino acids into muscle thus preserving body proteins. Another indirect, metabolic role of GH is its ability to stimulate the diversion of glucose and fatty acids away from tissue deposition, therefore making them available for energy needs (Raben, 1973; Trenkle, 1981). High serum GH

concentrations are observed in heifers at parturition (Ingalls et al., 1973). This elevation in GH is attributed to changes in the body's metabolism to meet the increased demand for energy and protein for the initiation of lactation (Convey, 1974). Circulating GH concentrations are high from the time of parturition until peak lactation, approximately 8 weeks postpartum, and then decline throughout lactation reaching their lowest concentration during the dry period (Convey, 1974).

Exogenous GH will increase yield in cattle (Asminov et al., 1937; Brumby et al., 1955) when administered during lactation both in the short- and long-term (Hart et al., 1978). Kazmer et al. (1986) and Bonczek et al. (1988) found greater circulating GH concentrations in Holstein cows selected for increased yield compared to controls, findings that confirm a trend observed in the much smaller trial of Flux et al. (1984) using New Zealand Friesians. Kazmer et al. (1986) also found that cows with higher genetic potential for milk yield were able to secrete more GH in response to a thyrotropin releasing hormone (TRH) challenge than the control cows. Subsequently, Lukes et al. (1989) found that a similar differential release was evident using growth hormone releasing factor (GRF) 45 days after parturition but not at parturition itself. These findings suggest that GH and GH secretion could have a role in defining the physiological attributes of genetic merit for milk yield. However, observations on groups of lactating cows may be confounded, since concurrent differences in energy balance due to

differing lactational performance may exist (Wooliams et al., 1991). When genetically different juvenile cattle were compared, Barnes et al., (1985) found female Holsteins selected for increased milk yield had greater concentrations of GH than controls at 6 and 12 months of age both before and after feeding, but not at 18 months of age. Mackenzie et al. (1988) observed greater concentrations of GH iin high genetic merit New Zealand Friesian calves both during fasting and after refeeding than in low merit counterparts. Conversely, Land et al., (1983) found that HerefordXFriesian calves had greater GH concentrations than Friesian calves during fasting, and neither Land et al.(date) nor Williams (date)found any differences before or after normal feeding or during fasting and after refeeding when sampling Friesian calves of differing merit.

An important difference between high- and low-yielding cows is the priority given to the mammary gland and the body tissues in the distribution of nutrients. Associations between milk yield and plasma concentrations of GH, both temporally throughout lactation, and between cows, suggest a galactotrophic effect of this hormone in cattle (Hart et al., 1978). Chilliard (1989) conducted a study which supported this suggestion because of the fact that administration of exogenous GH of pituitary or recombinant origin consistently increased milk yield approximately 10 to 20 percent. Exogenous GH mediates changes in metabolism resembling those brought about by selection for increased milk yield (Peel et al., 1987). These findings suggest that variations in endogenous GH secretion may

mediate genetic differences in milk yield, although the association of endogenous GH with milk yield in lactating cows may be confounded by concurrent associations with net energy balance.

Growth hormone secretion is measurable in cattle of both sexes and before sexual maturity. The measurement of GH secretion requires frequent blood sampling because of its pulsatile secretion pattern (Anfinson et al., 1975). However, some information on pituitary responsiveness can be obtained by the administration of exogenous secretogues, a method used in diagnosing GH deficiency (Takano et al., 1984).

Growth hormone plays an important role in ensuring that nutrients are provided to the mammary gland for milk synthesis (Bauman et al., 1980). Observations that endogenous concentrations of GH are high in early lactation (Hart et al., 1980) and in genetically superior cows (Hart, 1983) are consistent with the concept that high GH concentrations are associated with the peak nutrient demands of the mammary gland. Short term administration of exogenous bovine GH increases milk yield in mid to late lactation (Peel et al., 1983). Two studies, one of 12 week's duration in low-yielding cows (Brumby et al., 1955), and another of 10 week's duration in late lactation (Machlin, 1973) reported increases in milk yield with no apparent difference in food intake or live-weight change.

Differences in plasma GH for both high- and low-yielding Hereford x Holstein cows were reported by Hart et al. (1975). Concentrations of GH were

high at peak lactation in Holstein cows selected for milk yield and decreased as lactation progressed. Whereas, in low-yielding cows, fed the same ration and thus consuming more energy in relation to their energy output in milk, plasma GH was lower. Therefore, it can be argued that the endocrine differences observed were caused by differences in energy balance and possibly are not the physiological expression of genetic difference for milk yield (Bauman and Currie, 1980; Bauman et al., 1985). In 1965, Moe investigated the effect of the level of intake on the utilization of diets by the dairy cow. Several years later, a study was conducted to calculate averages from the feed intake and energy utilization data of Moe (Bauman and Currie, 1980). It is accepted that early in lactation, dairy cows are producing more milk than their dietary energy intake allows for. Therefore, peak milk production precedes maximum dietary intake and cows are in a negative energy balance, mobilizing body tissue to meet energy demands. Bauman and Currie (1980) found that dietary intake did not meet energy demand of production until 16 weeks postpartum, at which point milk yield had fallen to less than 80% of peak production. High-yielding cows were found to be underfed and low-yielding cows overfed when fed the same quantity of ration, with similar energy intakes in both groups. Hart (1983) concluded that differences in plasma GH concentration between genetically selected high- and low- yielding cattle were due to differences in energy balance and not due to differences in genetic merit. In order to feed both groups to similar weight gains, high producers would have to

be fed ad libitum, while low producers would need to be restricted (Kazmer, 1986). This allows for differing and artificially induced physiological conditions that may well affect various metabolic relationships. Net energy balance (NEB) was reported, by Kazmer (1986) as being not different between cows of differing genetic merit for milk yield. The author concluded that observed differences in plasma GH could therefore not be attributed to differences in NEB. The obvious suggestion arising from this work was that increased plasma GH concentration was a physiological factor responsible for the improved milk yield of offspring of high Predicted Transmitting Ability (PTA) milk sires compared to herdmates from sires with lower PTA for milk production. Several authors have reported differences in GH concentration of cows with different breeding value within a breed (Bryant and Trigg, 1981; Davey et al., 1983; Flux et al., 1984; Barnes et al., 1985).

The galactopoietic activity of GH was reported by Cotes et al. (1949) and plasma GH concentration is positively associated with milk yields (Hart et al., 1979; Kazmer et al., 1986). Massri et al. (1985) took a different approach to relate plasma hormone concentration to performance traits such as lactation yield. The author investigated the magnitude of the pituitary release of GH following a growth hormone releasing factor (GRF) injection in calves of differing genetic merit for milk yield fed a high energy diet. The treatment group animals were challenged with a single injection of .1 ug/kg human pancreatic GRF (hpGRF 1-

40)NH_2. Human pancreatic GRF (1-44) and its fragments have been documented to cause elevated circulating concentrations of GH in the ruminant (Baile et al., 1983; Plouzek et al., 1983; McCutcheon et al., 1984; Mosely et al., 1984; Massri, 1985; Enright et al., 1986; Baile and Buonomo, 1987). Massri et al. (1985) found that the magnitude of the induced GH release did not reflect differences due to genetic potential for milk yield. Both groups of animals, control and select, responded with a peak GH concentration 10-20 minutes post injection and of similar magnitude. The author suggested that the high plane of nutrition could have masked any differences in pituitary responsiveness to GH release, noting the role of GH in fatty acid mobilization from adipose tissue in an energy deficient state. Selection calves were found to have greater basal GH concentrations than control calves. Similarly, Lovendahl et al., (1991) conducted an experiment investigating the endogenous GH release of dairy calves differing in their genetic merit for milk yield. Growth hormone secretion was induced by acute intravenous administration of GH-releasing factor at a dose of .2ug/kg body weight. The results of the trial provided evidence for a positive association of the release of GH following GRF administration with the predicted breeding value for milk yield. A clear association was evident between the magnitude of GH release and the concentration of GH prior to the administration of the secretagogue.

Since lactation is regulated by many hormones, some with opposing actions (Tucker, 1981; Bines and Hart, 1982), measurement of INS, GH, and Insulinlike

Growth Factor I (IGF-I) should be considered in studies aimed at developing a genetic merit index. Serjesen and Lovendahl (1986) suggest a physiological index of several hormones and metabolites as a more logical and possibly more reliable criteria for differentiating cattle on the basis of genetic merit for milk yield.

The metabolic action of INS with regard to nutrient utilization is primarily anabolic. Insulin stimulates glucose uptake and utilization by many peripheral tissues while inhibiting glucose synthesis and release from the liver (Bines and Hart, 1982). Proteolysis and lipolysis are inhibited by INS, while protein and lipid synthesis are stimulated (Basset, 1975). Through lactation the plasma INS concentration decreases initially and rises slowly after peak lactation (Herbein et al., 1985). Low-yielding (HerefordXFriesian) crossbred cows had higher INS concentration than high-yielding cows (Friesians) during lactation (Hart et al., 1978). This finding was confirmed by Bonczek et al. (1988) in Holstein cows where those selected for high yield had the lowest INS concentrations during peak and mid-lactation. However, during the dry period such differences disappeared (Bines et al., 1983). These finding have not been universal, since Lukes et al., (1989) found similar concentrations among genetic groups throughout lactation, and the smaller studies of Davey et al. (1983) and Barnes et al. (1985) found higher INS concentrations in cows selected for high yield. Higher concentrations of plasma INS were found in crossbred HerefordXFriesian calves than in purebred Friesian calves (Land et al., 1983). Within breed, in calves of selected

lines, this finding could not be verified (Land et al., 1983). In the studies of Barnes et al. (1985) and Mackenzie et al. (1988), the highest INS concentration was associated with high dairy merit. Further, Sinnett-Smith et al. (1987) found no differences in baseline INS in calves of high and low dairy merit.

To study the sensitivity of the pancreas to nutrient stimulation, the INS response to feeding and intravenous administration of glucose or propionate has been measured. Crossbred (HerefordXFriesian) calves showed a greater INS response to feeding than did purebred Friesian calves, and a greater INS response to feeding than did purebred Friesian calves. Similar differences were obtained when INS release was induced by propionate injection (Land et al., 1983). However, within breed, comparisons of INS release in high and low dairy merit calves did not reveal significant differences (Land et al., 1983; Sinnett-Smith et al., (1987). Xing et al. (1988) used arginine as an inducer of INS release in calves but no difference between genetic group s was detected. Although a greater INS response to glucose was found in high breeding index calves in the study of Mackenzie et al. (1988), equivalent difference was also observed in the baseline concentrations in that experiment which may have had a contributing influence. Thus, the sensitivity of the pancreas to nutrients does not appear to be a clear indicator of dairy merit, under the conditions used in these experiments. The sensitivity of the peripheral tissue to the action of INS is a part of this axis, and may be measured as the change in plasma glucose and free fatty

acids following administration of INS. An increased glucose clearance in response to INS was found in high breeding index bull calves (Mackenzie et al., 1988) but this was not found in the study of Barnes et al. (1985) nor in the larger study of Land et al.. (1983).

Involvement of INS with processes which divert energy toward body tissue and away from milk synthesis is high (Yang and Baldwin, 1973). Treatment of lactating cows with INS causes an immediate decrease in milk yield which can be reversed by infusing glucose (Kronfeld, 1963). Changes in body weight in dairy cattle are positively correlated with similar changes in plasma INS concentration. Rabinowitz et al. (1966) investigated the effect of changes in the GH:INS ratio on fat mobilization. The authors proposed that in the human, human GH (hGH) potentially has both an anabolic role, for example, promotion of protein synthesis, and a diabetogenic role, for example, promotion of hyperglycemia and fat mobilization. The hypothesis was that the presence of INS maximized the anabolic actions of hGH, and conversely in the presence of very low or absent levels of INS, the diabetogenic actions of hGH are greatest.

Injection of GH into lactating ruminants increases milk yield by 10-40% (Collier et al., 1984). While the mechanism of action of GH remains to be elucidated, it has been established that GH does not achieve its effects through a direct action on the udder. The evidence is as follows: there was no direct effect of GH on milk synthesis in bovine mammary tissue in vitro (Gertler et al., 1983);

unilateral close-arterial infusion of GH into the udder of lactating ewes did not induce a differential response in milk yield between each udder-half (McDowell et al., 1987); specific binding of bovine GH to mammary membranes from lactating ewes or cows was not shown (Akers, 1985). Therefore it is likely that the galactopoietic effect of GH requires mediation of other factors. Insulin-like growth factor I is a candidate for such a role because of its GH dependence (Gluckman et al., 1987). Increased plasma concentrations of IGF-I have been observed in lactating cows treated with GH (Davis et al., 1987) and some in-vitro (Baumrucker, 1985) and in-vivo evidence supports a role for IGF-I in galactopoiesis. It is not known what controls IGF-I levels in milk or mammary tissue. Insulin-like Growth Factor I may be locally produced, but the absence of significant binding of bGH in mammary tissue suggests that its production must be controlled by key factors other than GH (Akers, 1985; Keys et al., 1988). Alternatively, if the major source of IGF-I was its transfer from blood, absolute concentration in plasma would appear to have only minimal influence on tissue concentrations (Prosser et al., 1991).

Insulin-like growth factor I is an important mediator of the biological effects of growth hormone. Its growth promoting action has been demonstrated in vivo in hypophysectomized (Zapf et al., 1987) and diabetogenic rats (Scheiwiler et al., 1986) and it was proposed to mediate the positive action of GH on milk production in dairy cows (Bauman et al., 1986). Bovine IGF-I has been purified

and characterized: this 70 amino acid peptide is identical to human IGF-I (Honegger et al., 1986). In bovine serum, as in other species, IGF-I is bound to large molecular weight binding proteins. It is mainly associated with a 145 kD IGF binding protein, which is GH dependent, and minor quantities are associated with a 35-39 kD protein (Hossner et al., 1988).

Originally, the somatomedin hypothesis suggested that GH, exerted its effects by stimulating IGF-I release from the liver which then mediated the somatogenic actions in the target tissues (Salmon & Daughaday, 1957). Subsequently, it has been shown that IGF-I is produced within many tissues in addition to the liver (D'Ercole, Stiles & Underwoood, 1984). This has led to a gradual erosion of the original concept and to the acceptance of an alternative concept of IGF-I action, with GH considered to act by stimulating IGF-I production in target tissues which then acts in an autocrine or paracrine manner on local cells (Underwood et al., 1986).

The importance of circulating IGF-I in animal growth and lactation is not clearly defined. With respect to lactation, four lines of evidence suggest an important regulatory role for circulating IGF-I. Firstly, elevated serum IGF-I concentration is observed in conjunction with the enhanced lactational performance of bovine somatotropin (bST)- treated-dairy cows (Peel et al., 1985; Davis et al., 1987; Cohick et al., 1989). Secondly, it has been shown that a large proportion of exogenously infused IGF-I is rapidly transferred into mammary

lymph of lactation sheep (Hodgkinson et al., 1990). Also, using an immunohistochemical technique, Glimm et al. (1988) have shown that bST treatment of dairy cows causes an increase in immunoreactive IGF-I in mammary tissue in general and in the cytoplasm of mammary epithelial cells in particular. Third, the type I IGF receptor has been characterized in bovine mammary tissue (Campbell and Baumrucker, 1986; Dehoff et al., 1988) and in-vitro studies have shown that IGF-I will stimulate DNA synthesis in ovine mammary epithelial cells (Winer et al. 1989) and in explants of bovine mammary tissue (Baumrucker and Stemberger, 1989). Lastly, the lactational effects of bST appear to be mediated by a second factor such as bST receptors which appear to be absent from the bovine mammary gland (Gertler et al., 1984). However, this point has been challenged because of the reported presence of mRNA for the bST receptor in the bovine mammary gland (Glimm et al.,1990; Krivi et al.,1990). This finding suggests a direct bST effect mediated through local IGF production.

Kerr et al. (1991) generated profiles of circulating IGF-I levels for lactating dairy cows and related these profiles to milk yield. Differences in high- and low- producing cows were not reflected in differences in serum IGF-I concentrations. Also, the greater milk production of cows as compared to heifers occurred despite lower IGF-I levels in the older animals. Furthermore, as milk yield declined during lactation, IGF-I levels increased. Similar findings were reported by Ronge et al. (1988). In addition to negative correlations between

IGF-I and milk yield, Ronge et al. described a positive relationship between serum IGF-I concentrations and net energy balance. Other studies in ruminants (Breier et al., 1986; Brier et al. 1988; Rutter et al, 1989) have documented the decline in serum IGF-I levels which occurs during periods of experimentally induced reductions in energy balance. In-vitro studies have demonstrated mitogenic and galactopoietic effects of IGF-I in bovine mammary tissue (Baumrucker 1986; Baumrucker and Stemberger 1989). Arbitat et al. (1990) conducted a study to evaluate variations of plasma IGF-I concentration with age and stage of lactation and following administration of GRF in Holstein cows. The authors found IGF-I concentrations to be high during the dry period and markedly decreased 24 hr after calving. The IGF-I concentration following an acute GRF injection (10ug/kg) increased, and was maximal 8 to 10 hours after the GRF injection. This time lag to peak IGF-I has previously been observed in cattle after GH administration (Elasser et al., 1987; Cohick et al., 1987; Kerr et al., 1988). This observation supports the hypothesis that, similarly to exogenous GH, GRF- induced GH secretion induces de novo synthesis of IGF-I rather than causing its release from storage pools (Wilson et al., 1985). This was also demonstrated by an increase in IGF-I mRNA in liver of genetically GH-deficient mice following GH administration (Mathews et al., 1986). Marked elevations in serum IGF-I and milk production occur concurrently in bST or GRF treated cows (Peel et al. 1985; Davis et al. 1987; Cohick et al. 1989; Arbitat et al. 1990). These

findings and the reported lack of bST receptors on the bovine mammary gland (Gertler et al. 1984) suggest an important endocrine role for IGF-I in the control of lactation.

CHAPTER I

INTRODUCTION

Recent work has shown that increasing selection pressure for milk yield in dairy cattle increases the efficiency of production, and this increase is likely associated with increased growth hormone (GH) secretion (Barnes et al., 1990; Kazmer et al., 1986). Evidence indicates that GH is galactopoietic but suggests that its action on the mammary gland is likely mediated through increased production of insulin-like growth factor (IGF-I) (Prosser et al., 1991).

Insulin-like growth factor is a potent GH dependent mitogen that has been shown to mediate many of the somatogenic effects of GH (Hammond et al., 1990). These actions and the role of IGF-I in regulation of growth have been reviewed (Daughaday et al., 1989). While some of the actions of IGF-I may be paracrine or autocrine in nature, circulating concentrations of IGF-I are related to growth rate, nitrogen balance and body size in adult animals. Circulating concentrations of IGF-I are dependent on GH, thus elevated endogenous GH secretion may be associated with elevated production of IGF-I in dairy cattle. Since GH secretion has been documented to decrease with stage of lactation in dairy cows (Koprowski et al., 1973; Smith et al., 1975) it is relevant to evaluate the variations of IGF-I concentrations in

relation to stage of lactation.

In view of the role of IGF-I in mediating some of the endocrine effects of GH, this study was designed to examine whether the galactopoietic actions of GH in dairy cows of differing genetic merit were accompanied by changes in plasma concentrations of IGF-I during first lactation.

Materials and Methods

Twenty-two primiparous Holstein cows were housed in a free stall barn at the Virginia Tech Dairy Center and milked twice daily in a milking parlor. Animals were either daughters of cows bred to fifth generation daughters of cows continuously randomly bred to non-selected sires originating in the Virginia Tech dairy herd (control group, n=10) (Mean estimated sire Predicted Transmitting Ability (PTA_{90})=-678 kg) or to daughters of cows bred to selected, commercially available artificial insemination (AI) sires (selection group, n=12) (weighted mean sire Predicted Transmitting Ability (PTA_{90}=+681 kg). The estimated genetic difference in transmitting ability for the 2 groups of bulls used to sire these cows was 1359 kg milk. Daily milk yield for both morning (a.m.) and afternoon (p.m.) milkings was monitored and recorded for each cow. Phenotypic performance during the first lactation of these cattle resulted in a difference 2577 kg milk, with selection cows producing 8387 kg milk, and control cows producing 5810 kg milk.

All animals were fed the same mixed complete diet (corn silage, haylage and concentrate) ad libitum during all stages of lactation. The ration averaged 55% dry matter (DM), 16% crude protein(CP) and 1.56 Mcal NE/kg on dry matter basis. Body weight (BW) was recorded biweekly. Body weight was converted to metabolic body weight (MBW = $BW^{.75}$) for use in energy balance calculations. Data were

collected throughout the first lactation. The experiment was begun September, 1990 and completed October, 1991.

Serial blood samples were collected at 30d, 60d, 90d, 120d, 150d, 180d, 210d, 240d, 270d and 300d postpartum (DPP) via jugular cannulae inserted 3.5 h prior to sampling. Samples were collected into tubes containing 200 ul of a disodium ethylenediamine tetraacetate solution (60 mg per ml saline Na_2 EDTA, Fisher) at 15 min intervals from 0900 to 1400 h. Cows were milked at 0300 h and 1500 h on the sampling day. Blood was immediately chilled on ice and then centrifuged (3,000 x g) for 20 min and plasma was stored at -20 C until assayed for GH or INS content.

Feed intake data were recorded for 5 consecutive days centered at 45 d intervals via an electronic intake monitoring device (Pinpointer, Inc.) at 45, 90, 135, 180, 225, 270 and 315 DPP. Milk yield during the 5 d feed intake data collection period was recorded at each milking. Net energy balance was calculated using the NRC Recommendations (1989) at 45, 90, 135, 180, 225, 270, and 315 DPP. To calculate net energy balance (NEB), dry matter feed intake (DMI), BW and milk yield were used in conjunction with the National Research Council (NRC,1989) factors concerning energy requirements for maintenance and milk yield. The appropriate factors from NRC tables were multiplied by metabolic BW ($BW^{.75}$) during each sampling period to yield the energy requirement for maintenance (energy requirement for maintenance = .08 Mcal NE/kg*$BW.^{75}$). Energy requirement for milk yield was calculated by multiplying the appropriate NRC factor

by the fat percent of the milk as well as the kg of milk produced. Milk fat test data were obtained from Dairy Herd Improvement Association (DHIA) records, using the data from the test day occurring in the same month as the sampling period. Total energy was then calculated as the sum of the requirement for maintenance and the requirement for milk yield. Energy intake was calculated by multiplying the energy content (Mcal/kg DM) of the ration by the daily amount consumed. Finally, NEB was calculated as the difference between energy intake and the total energy requirement.

PLASMA HORMONE CONCENTRATIONS

Plasma GH concentration was quantified using a double antibody RIA by the method of Barnes et al. (1985). The specific antibody (100 ul) used at an initial dilution of 1:1000, bound approximately 50% of labelled GH in the absence of unlabelled hormone (final volume = 800 ul). Samples volumes of 300 ul plasma were assayed in duplicate and the intra- and inter-assay coefficients of variation averaged 6% and 9% respectively, in the plasma pools.

Plasma INS concentration was quantified using a double antibody RIA by the method of Barnes et al. (1985). Sample volumes of 300 ul plasma were assayed in duplicate and the intra and inter-assay coefficients of variation averaged 8% and 10% respectively, in the plasma pools.

Plasma IGF concentration was quantified using a double antibody RIA by the method of McFadden et al. (in press). The specific antibody used an initial dilution

of 1:9000, bound approximately 20% of labelled IGF-I in the absence of unlabelled hormone. Sample volumes of 50 ul plasma, were assayed in duplicate and the intra- and inter-assay coefficients of variation averaged 5% and 10% respectively, in the plasma pools. The standard ranged from 20 pg to 1.25 ng.

Data Analysis:

Hormonal data were analyzed using the General Linear Models (GLM) option of the Statistical Analysis System (SAS, 1982). Blood hormones were analyzed using a model accounting for genetic selection group, cow(genetic selection group), DPP, DPP X genetic selection group and DPP X cow(genetic selection group). The mean square for error was used to test all other effects. Metabolic body weight, DMI, NEB, and ratio DMI/MY (Milk Yield) were analyzed using a reduced model. (See Appendix A, Model 1)

RESULTS

Selection cattle had greater ($P<.05$) milk yields (8387 kg milk) than control cattle (5810 kg milk) (Figure 1). Selection cows averaged 294 days in milk (DIM) while control cows averaged 253 DIM. Mean milk fat yield was higher for selection group cows than for controls (.96 vs .78 kg/d, $P<.01$) (Figure 2).

Selection group cattle had higher mean energy intake (EI) (28.6 vs 25.6 Mcal/d, $P<.05$) than did control cows (Figure 3). Energy intake increased until 135 DPP for selection group cows and then decreased to 180 DPP where it remained constant for the remainder of the lactation. Control group cattle decreased EI from 90 DPP to 135 DPP and continued at that level until a second decrease in EI occurred at 225 DPP. Energy intake then continued to decline steadily until the end of lactation in control cows.

Control and selection group cows had mean BW of 514 vs 532 kg, respectively (Figure 4). A group by DIM interaction ($P<.01$) occurred because selection group cattle weighed more after calving, and lost weight until 6 weeks of lactation, while control group cows gained weight steadily from 2 weeks postpartum until the end of lactation. Cows in the selection group took 16 weeks to regain a mean BW similar to that of mean calving weight.

Mean estimated production efficiency (conversion of feed to milk;kg milk/Mcal intake) was greater for selection group cows (1.02 vs .84 kg milk/Mcal Intake, $P<.05$) than for control cows (Figure 5). Production efficiency ratios were

Figure 1. Mean Milk Yield For First Lactation Control and Selection Group Holstein Cows

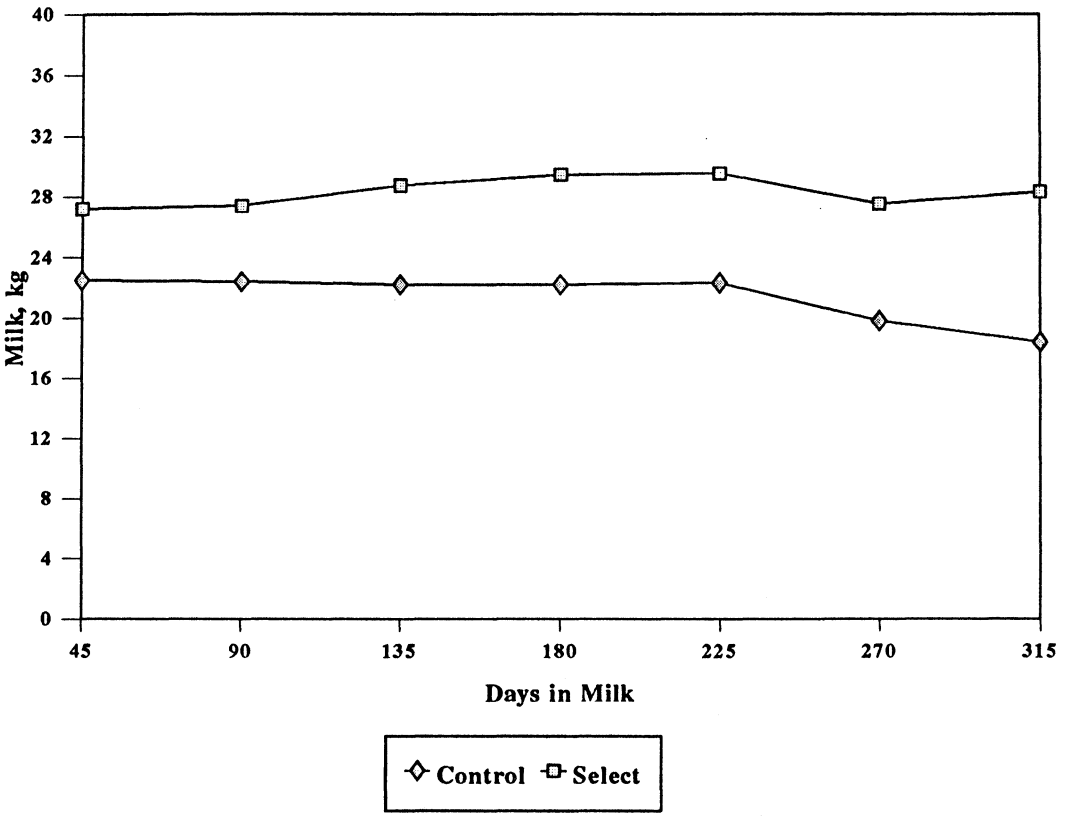


Figure 2. Mean Milk Fat Yield For First Lactation Control and Selection Group Holstein Cows

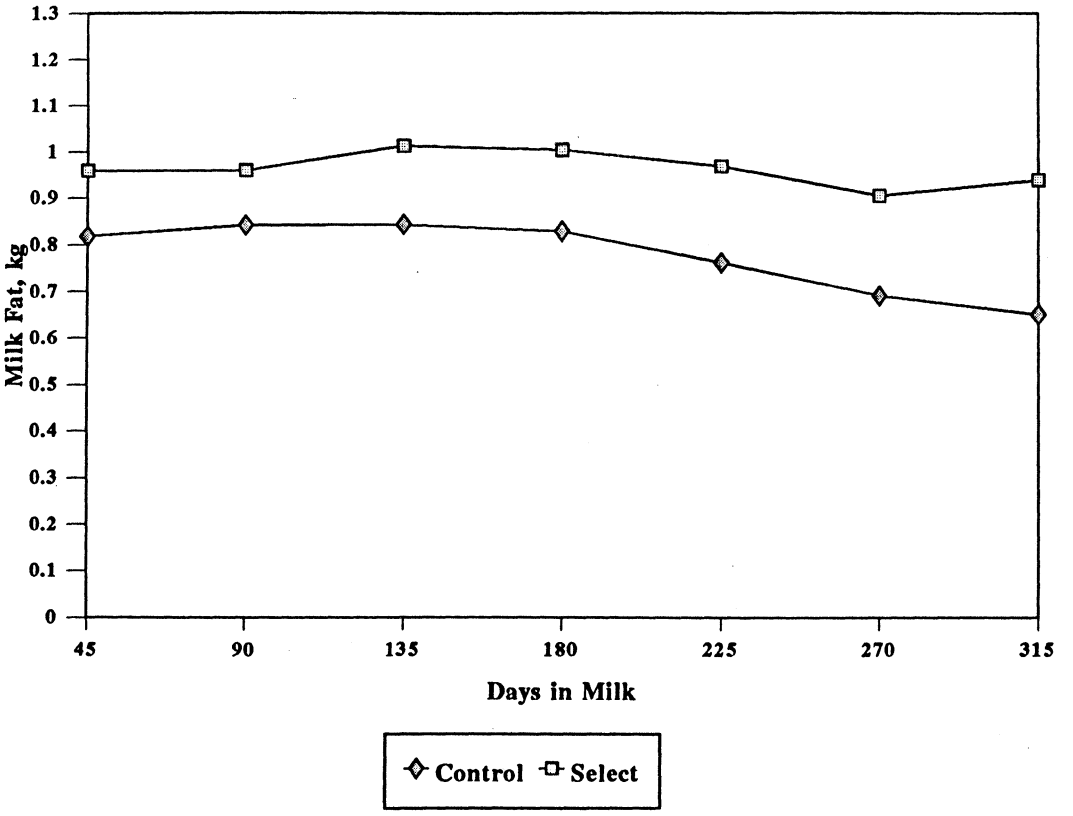


Figure 3. Mean Energy Intake for First Lactation Control and Selection Group Holstein Cows

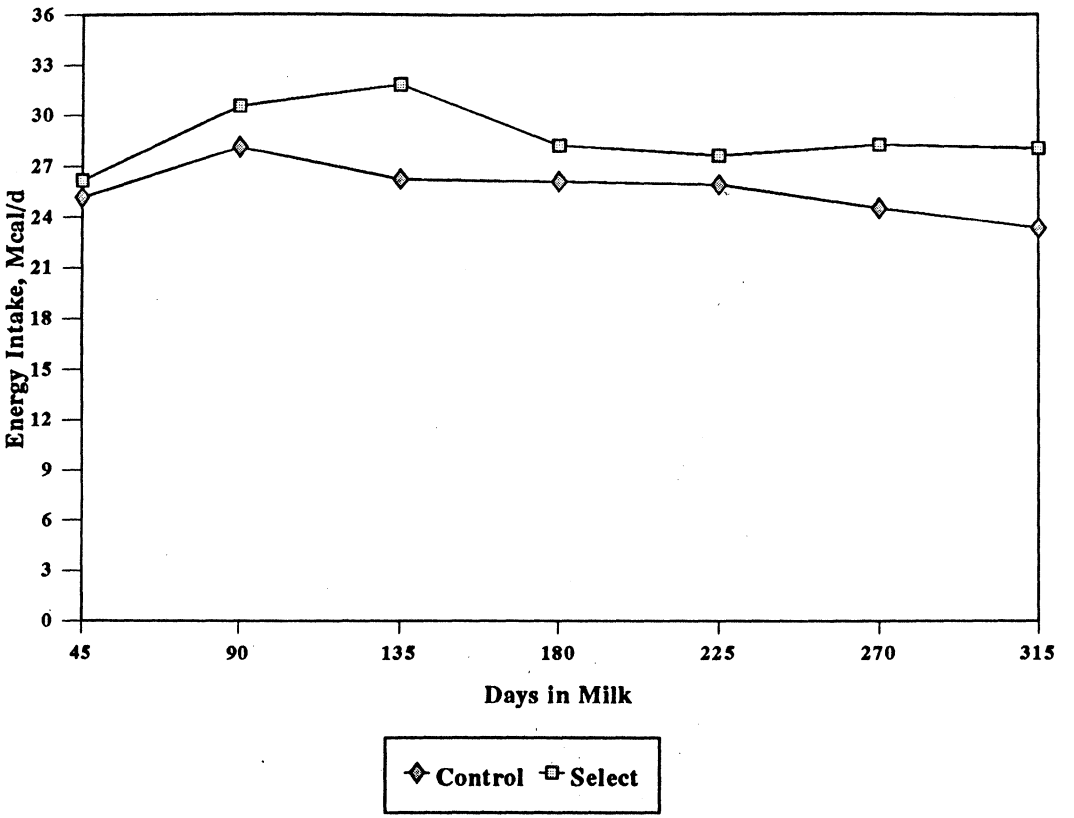


Figure 4. Mean Body Weights for First Lactation Control and Selection Group Holstein Cows

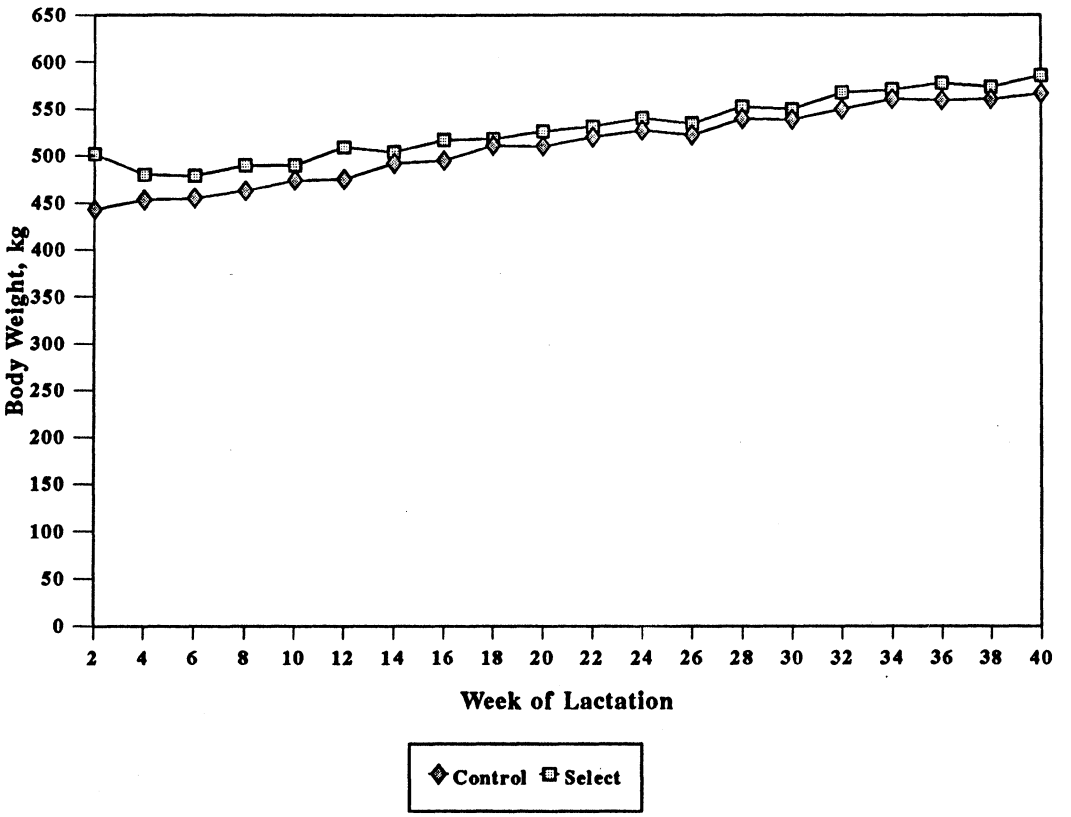
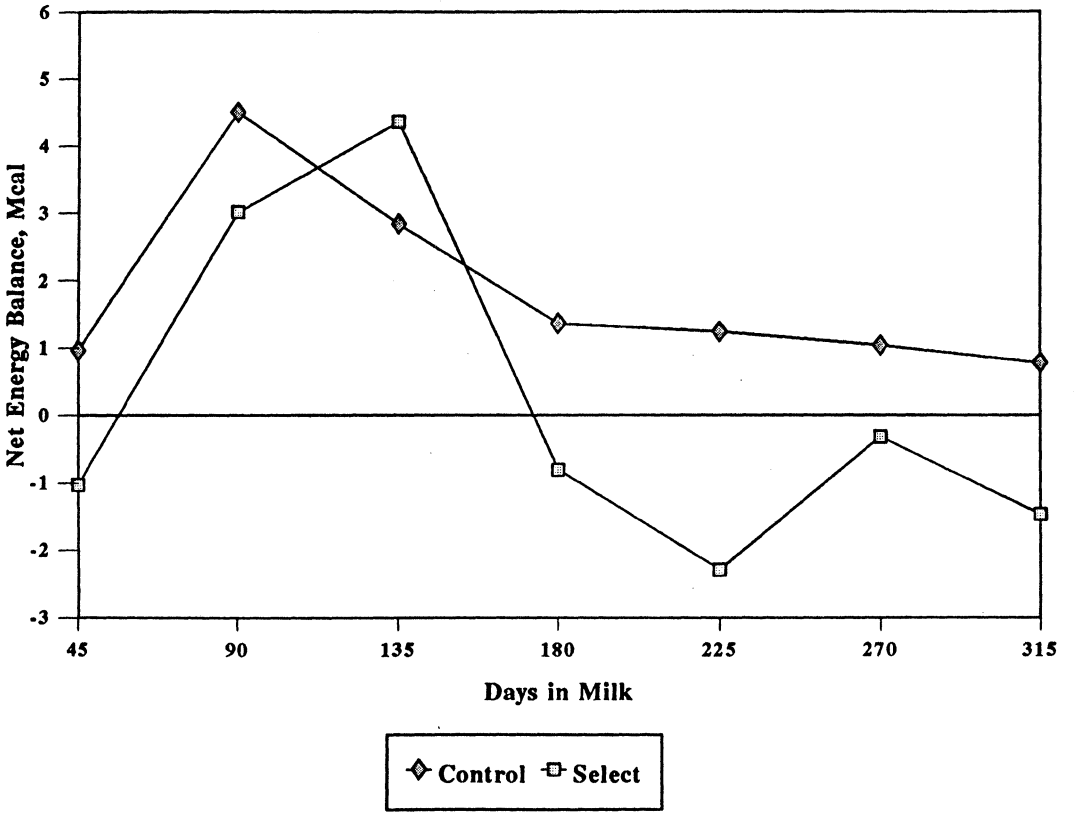


Figure 5. Mean Net Energy Balance for First Lactation Control and Selection Group Holstein Cows



similar in both groups through 135 DPP. The greatest ratio differences between the two groups were at 225 and 315 dpp, when the ratios of kg milk/Mcal Intake were 1.1 vs .5 and 1.0 vs .3 for the selection and control groups, respectively.

Control group cattle maintained more positive mean NEB (1.82 vs .33 Mcal, N.S.) than selection cows (Figure 6). At 45 DPP, selection group animals were in negative NEB balance (-1.03 Mcal), whereas control animals maintained positive NEB at those periods measured during lactation. At 90 DPP selection cows were in a less positive NEB than control group cows (2.70 vs 4.5 Mcal). Selection group cows shifted back into negative NEB between 135 DPP and 180 DPP and remained in negative NEB for the remainder of the lactation. Control group cows declined to a less positive NEB after 90 DPP until 180 DPP, when NEB was maintained at a constant level until drying off.

Mean plasma GH was higher overall (15.2 vs 10.4 ng/ml, $P<.01$) for selection compared to control group cows (Figure 7). Basal plasma GH concentration decreased in both groups with advancing lactation. Basal concentrations of plasma GH were greatest at 30 DPP, and lowest at 300 DPP in both groups. Mean plasma INS concentrations were 821 vs 763 pg/ml for control and selection group cows (N.S.) (Figure 8). Selection group cattle showed a decline in INS concentration as lactation progressed, while control animals showed inconsistent increases and decreases through 240 DPP. However, control group cows showed a steady increase in INS concentration from 240 DPP forward, which was not observed in the selection

Figure 6. Mean Estimated Production Efficiency (kg milk/Mcal EI) for First Lactation Control and Selection Group Holstein Cows

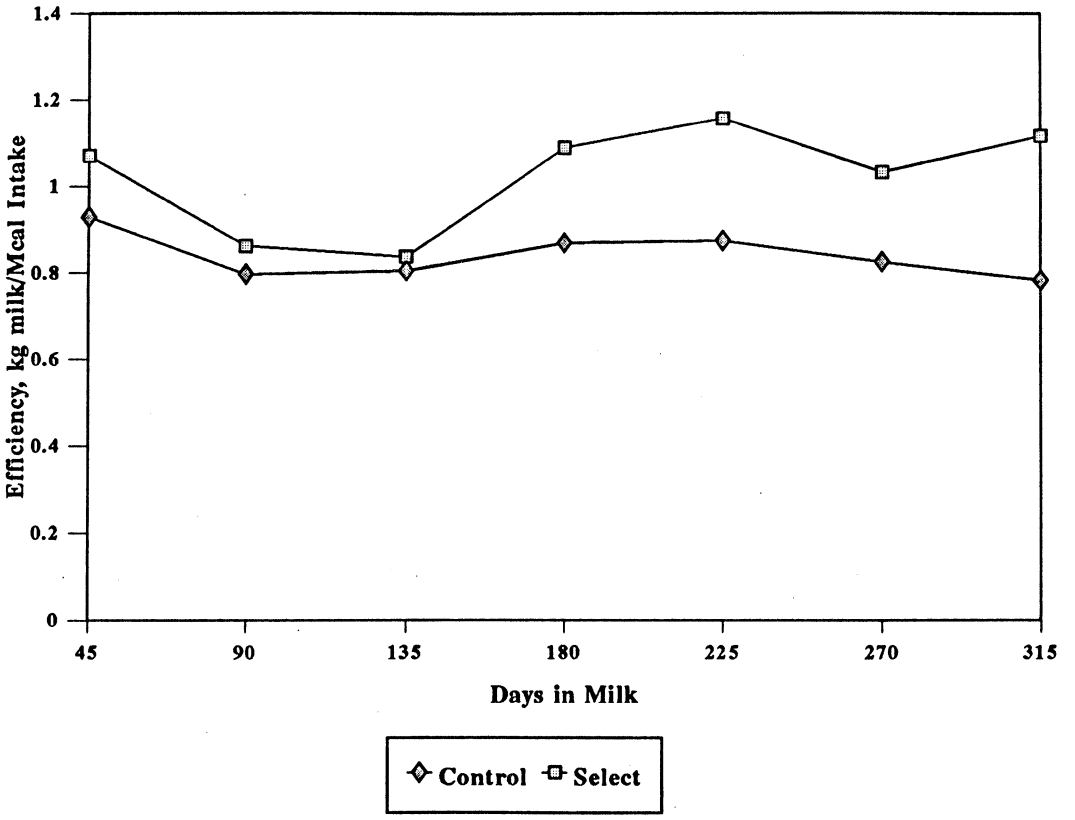


Figure 7. Mean Plasma Growth Hormone Concentrations (ng/ml) for First Lactation Control and Selection Group Holstein Cows

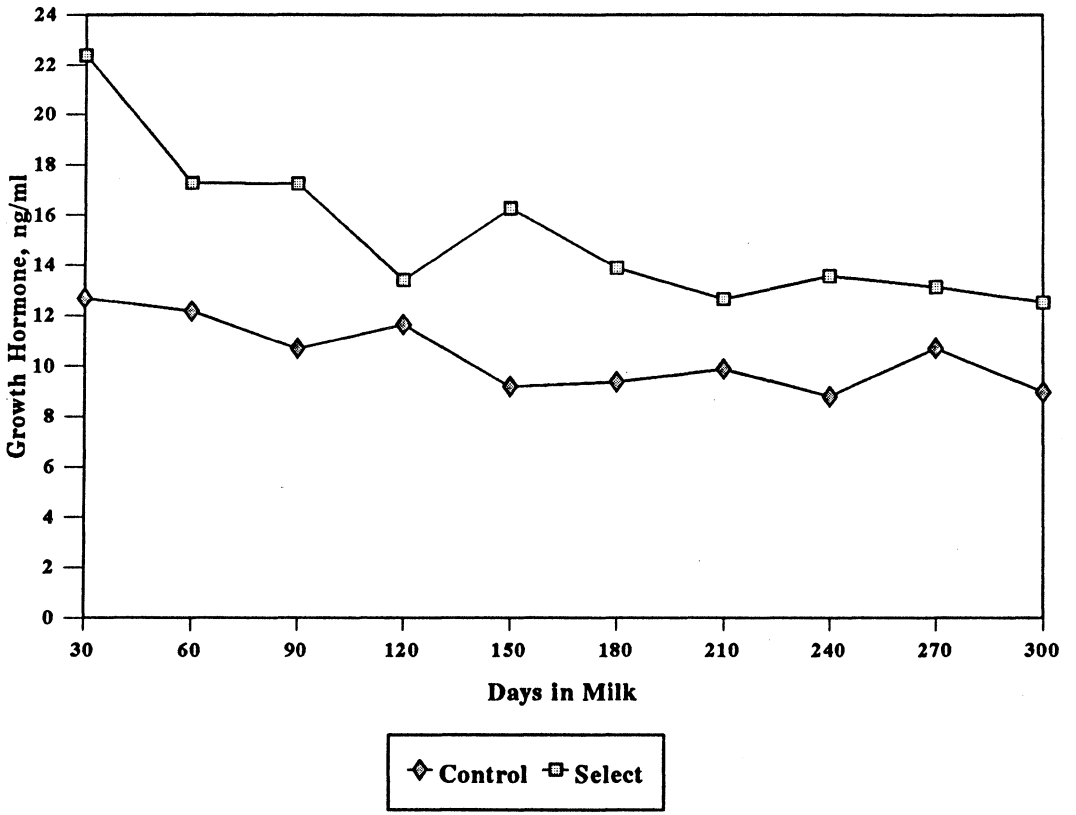
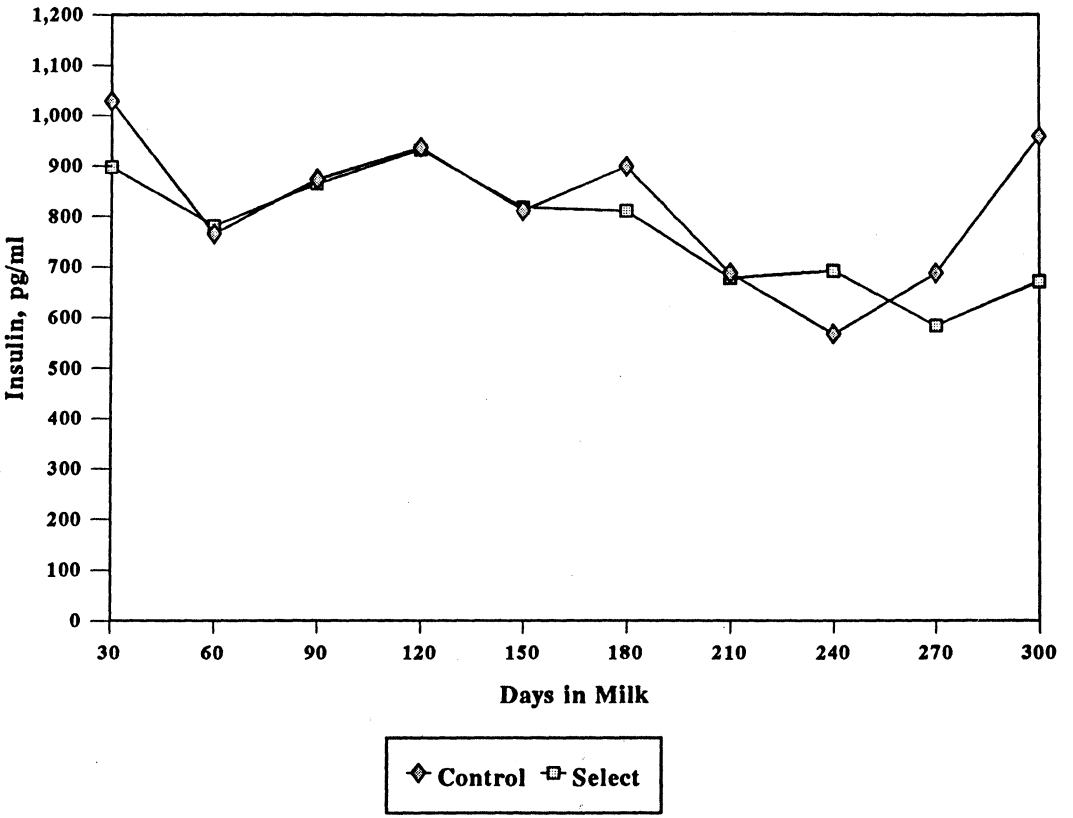
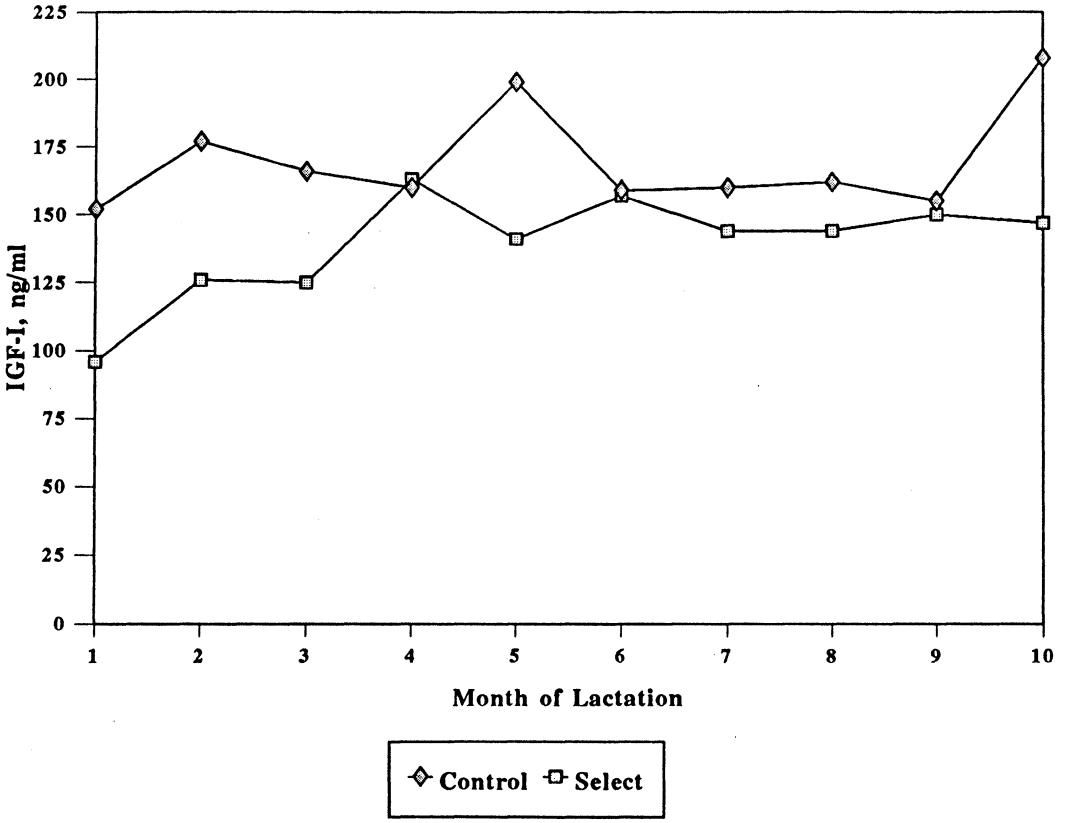


Figure 8. Mean Plasma Insulin Concentrations (pg/ml) for First Lactation Control and Selection Group Holstein Cows



cows. The control group tended ($P<.1$) to have higher mean plasma IGF-I concentrations than the selection group. Mean IGF-I values were 170 and 139 ng/ml for the control and selection group cows, respectively. Control groups cows consistently had IGF-I levels either similar to or greater than selection group cows during the entire lactation.

Figure 9. Mean Plasma Insulin-like Growth Factor I (IGF-I) (ng/ml) Concentrations for First Lactation Control and Selection Group Holstein Cows



DISCUSSION

An increased level of milk yield in the selection group cows as compared to the control group animals was observed. Selection group cows in the present study were characterized as more efficient producers especially in late lactation. From 180 DPP forward selection cows were 75 % more efficient, because they consumed similar energy intake, while yielding more milk compared to control cows. Bryant and Trigg (1981) also reported that cows of higher genetic merit for milk yield were more efficient producers in terms of utilizing feed intake for milk yield. Interestingly, selection group animals did not reach peak milk yield until 225 DPP, at which point they were producing almost 30 kg/d. The lactation curve for these cows, as well as for control group cows is relatively flat, and for both groups yield was maintained at a level close to peak yield throughout lactation. It is difficult to hypothesize why selection and control group cows maintained relatively flat lactation curves. Even though control group cattle yielded significantly less milk when compared to selection group cows, as a group they maintained a yield of 22 kg/d, during early, mid and late lactation. One cause for the results seen in this study may be attributable to a change in the group's diet. At the onset of this study both groups were receiving the same total mixed ration fed ad libitum, consisting of a combination of corn silage, haylage and concentrate. After approximately six months into this trial the diet was reformulated to include whole cottonseed as an additional energy source. The new diet was calculated to be 52% DM, 16% CP, and NE_L remained at 1.6 Mcal. Both

selection group and control group cows received this same newly formulated diet ad libitum as previously fed. Whole cottonseed is a high-fat, high-energy, and high-protein feedstuff and has been fed experimentally to dairy cows at .81 to 6.35 (Smith et al., 1988) kg daily. Some, but not all, research indicates increases of milk production and milk fat percentage by cows fed diets containing whole cottonseed (Davis et al., 1946; Stanley et al., 1969; Tomlinson et al., 1981). This additional energy source may have provided selection group animals with the necessary milk precursors to increase yield thereby causing peak milk yield to occur late in lactation. The control group cows may not have been able to sufficiently metabolize this additional energy from the whole cottonseed in a way which increased milk yield. Since mean plasma INS tended to be greater in control cows the level of EI may have been responsible for their ability to maintain a high level of persistency throughout lactation. However, the increased ratio of INS to GH in these cows may have promoted greater utilization of nutrients for maintainence rather than milk synthesis.

Energy intake decreased greatly in the selection group cows at approximately the same time that the addition of the whole cottonseed to the diet occurred. It is possible that the new diet was not as palatable to selection group cows, however this seems unlikely because no apparent difference in the level of intake by control group cows was observed upon the addition of the whole cottonseed to the diet. While selection group cows decreased their level of intake, they continued to produce

increased milk yields, thus creating a negative NEB. Since these animals never regained an increased level of intake and continued to produce milk at levels near peak yield, selection group cows stayed in negative NEB for most of their lactation. Control group cows tended to maintain their level of intake and milk yield thus allowing them to remain in positive NEB for the duration of their lactation.

At calving selection group cows weighed more than control group cows which led to a significant group by DIM interaction. Selection group cows did not regain original calving weight until approximately sixteen weeks of lactation, whereas control group animals gained weight continuously throughout lactation. After the first sixteen weeks of lactation selection group animals gained weight at a rate similar to control group cows and reamained heavier. It is difficult to explain how selection group cows were able to increase milk yield and still gain weight despite a decrease in energy intake. Although there was an additional energy source added to the diet half way through the trial, it did not change the NE_L of the diet. It therefore does not seem likely that the whole cottonseed alone was responsible for the continuous weight gain and increased milk production accompanied by decreased energy intake of the selection group cows. This does support the fact that selection cows were more efficient, while control group cows may not have able to utilize the same energy substrates as effectively. Cummins and Sartin (1987) suggested that diets containing fat may have endocrine effects that are similar to those of positive energy balance, even when calculated energy intake is below requirements.

Several studies have shown that selection for milk yield is associated with increased plasma GH concentrations at several stages of lactation in dairy cows (Barnes et al., 1985; Kazmer et al., 1986; Bonczek et al, 1986; Bryant et al., 1981; Davey et al., 1983). In the present study selection group cows had significantly greater plasma GH concentrations than did control group cows. This is in accordance with Bines et al. (1982) who found high-yielding dairy cows have higher GH concentration than do low- yielding cows on similar dietary regimen. According to Convey (1974) circulating GH concentrations are elevated from the time of parturition until peak lactation, and then decline throughout lactation, reaching lowest concentrations during the dry period. In the present study, similar results were found in that GH was greatest early in lactation and tended to decrease as lactation progressed. However, selection group cows showed an increase in plasma GH concentration at approximately the same time the diet was altered, and the reduction in the level of energy intake occurred. The concentration of plasma GH did not remain elevated but instead it decreased as selection group cows declined further into negative NEB. Similarly, NEB became less positive for the control group animals late in lactation and this was accompanied by a decrease in plasma GH. Other researchers have suggested that differences in yield are related to higher levels of plasma GH accompanied by negative NEB in high producing animals as compared to low producers (Hart et al., 1978; Blum et al., 19851 Breier et al., 1986). However, in the present study selection group cows did not experience an increase

in plasma GH levels when experiencing negative NEB. Therefore, their ability to sustain increasing yields as lactation progressed enforces the theory that high-yielding dairy cows divert dietary energy to more efficiently attempt to meet the demands of the lactating mammary gland (Hart et al., 1979). Associations between milk yield and plasma concentrations of GH, both temporally throughout lactation, and between cows, suggest an galactotrophic effect of this hormone in cattle (Hart et al., 1978). This suggestion is supported by the fact that administration of exogenous GH of pituitary or recombinant origin consistently increases milk yield by approxiamately 10-20 % (Chilliard, 1989). Exogenous GH mediates changes in metabolism resembling those brought about by selection for increased milk yield (Peel et al., 1987) These findings suggest that variations in endogenous GH may mediate genetic differences in milk yield. However, the association of endogenous GH with milk yield in lactating cows is confounded by concurrent associations with energy balance.

Incorporation of animal or vegetable fats into dairy cattle diets increases caloric density of the diet without decreasing fiber (Cummins, 1987). Considerable research has been conducted on the effects of high fat diets on milk yield, fiber digestion and milk composition (Anderson et al., 1979; Devendra et al., 1974; Hawkins et al., 1985; Palmquist et al., 1981). Increased dietary fat decreases glucose pool volume and mass in dairy cattle (Palmquist et al., 1981) and rats (Lavau et al., 1979). Systematic effects of dietary fat in dairy cows have been documented (Palmquist et al., 1980; Palmquist et al. 1981). Smith et al. (1978) suggested that

excess dietary fat might alter glucose metabolism. In support of this concept, Palmquist and Moser (1981) observed a 25 % decrease in plasma INS when protected tallow was fed. Although the INS requirement for glucose uptake by the bovine mammary gland may be negligible (Kuhn et al., 1980), a specific role for INS in mammary protein synthesis has been proposed (Palmquist et al., 1981; Schmidt et al., 1966). Unlike nonruminats, ruminants depend on the microbial fermentation of nutrients and the interaction between dietary energy and protein influences on digestion of protein. Therefore both energy and protein utilization can alter the amount of nutrients presented for absorption in the gut (Elasser et al., 1988). Cattle in a greater positive NEB tend to have higher concentrations of INS and lower concentrations of GH than cattle in negative NEB (Bauman et al., 1979; Hart et al., 1978; Vasilatos et al., 1979; Vasilatos et al., 1981). Because of the greater energy density of whole cottonseed diets, it might be assumed that cows fed high fat diets would be in a more positive NEB. In the present study while both groups received the same diet, the control group cows were in more positive NEB than the selection group cows. Plasma INS concentrations did not follow the expected pattern of increase throughout lactation for either group. Although not significant, control group cows had slightly greater plasma INS concentrations and began to show a sharp increase in plasma INS concentrations later in lactation. However, selection group cows showed a continuous decrease in plasma INS concentration beginning at 120 DIM and throughout lactation, reaching its lowest point at 270 DPP. Thus, it

appears that declining NEB had a depressing effect on INS secretion. The literature offers a possible explanation such that if energy balance is positive, plasma INS concentrations tend to be higher and plasma GH lower (Bauman et al., 1979; Hart et al., 1978; Vasilatos et al., 1979; Vasilatos et al., 1981). Thus it would seem to follow that when energy balance is negative then plasma INS would decrease accordingly. This would help explain the phenomenon observed in selection group cattle whose energy balance was negative during lactation, especially during mid-late lactation when GH stayed relatively constant, but INS decreased. Meanwhile control group cattle were in a positive energy balance, with lower GH levels and higher INS concentrations. Again, the increased ratio of circulating GH to INS in selection cows as compared to controls supports the fact that the selection cattle were more efficient and yielded more milk than controls.

Evidence in support of a relationship between basal IGF-I concentrations and lactation level was not generated in this study. Differences between high- and low-yielding cows were not reflected in differences in plasma IGF-I concentrations. Also, the greater milk yield of selection group cows as compared to the control group occurred despite lower IGF-I concentrations in selection group cattle. Similar findings have recently been reported by Kerr et al. (1991). In addition to negative correlations with milk yield, Ronge et al., (1988) described a positive relationship between serum IGF-I concentrations and NEB. In the present study control group cows consistently had higher plasma IGF-I concentrations and positive NEB, while

selection group cattle were in a much reduced NEB with lower plasma IGF-I concentrations. Other studies in ruminants (Breier et al. 1986; Breier et al., 1988; Rutter et al., 1989) have also documented the decline in serum IGF-I levels which occurs during periods of experimentally induced reductions in NEB. Spicer et al. (1990) demonstrated an increase in NEB associated with an increase in concentrations of IGF-I in serum during early lactation and increased milk yield is associated with decreased serum IGF-I concentrations and NEB. Spicer et al. (1990) revealed an inverse relationship between milk production and IGF-I secretion. Ronge et al. (1988) observed a negative correlation between milk production and IGF-I secretion. In vitro studies have demonstrated a direct stimulatory effect of IGF-I on bovine mammary growth with no effect on alpha lactalbumin production (Shamay et al. 1988). This suggests that IGF-I may stimulate mammary growth but have no effect on milk synthesis. Why milk production would increase in the presence of lowered IGF-I is unclear but may be due to changes in other galactopoietic hormones. A similar relationship to milk production has been documented for INS (Koprowski et al., 1973; Sartin et al., 1988; Tucker et al., 1981) and may be related to removal of the barrier to partition nutrients away from the mammary gland. As aforementioned IGF-I in blood appears to be influenced by nutritional status of the animal, and cows with higher milk yields are under greater nutritonal stress (i.e., negative NEB) than cows with lower milk yields (Ronge et al. 1988; Villa-Godoy et al., 1988). Certainly, no single hormone is the sole regulator

of lactation, but rather a complex of hormones likely interplays to determine lactational intensity (Akers et al., 1985; Bauman et al., 1980; Collier et al., 1984).

CONCLUSIONS

The results indicate that selection for milk yield results in more efficient production (milk kg/Mcal intake) and differences in EI, plasma GH and IGF-I concentrations. Consistent effects of selection on blood INS and BW were not evident under the conditions of this study, indicating no uniform direct effect of selection pressure on regulation of this hormone. Higher plasma IGF-I concentrations associated with the animals in more positive NEB, in this case the control group cattle, is consistent with results of Spicer et al. (1990).

In summary, plasma IGF-I concentrations appear to be of little value in predicting the lactational performance of dairy cattle. It is possible that plasma concentrations of IGF-I are only a poor reflection of physiologically more important IGF-I synthesis at the local tissue level.

CHAPTER 2

INTRODUCTION

Understanding the function of the mammary gland is central to any efforts to improve dairy cattle production. Hormones are the primary physiological factors that stimulate mammary growth and initiate and maintain lactation (Takami et al., 1991). This topic is of importance because manipulation of the endocrine system is a potential method to improve efficiency of milk yield. More than thirty years ago Reece (1955) summarized research contributions in the field of mammary physiology. Since then, substantial progress has been gained toward understanding the physiological control of the mammary gland.

Hormonal supplementation is an attractive method to improve the efficiency of livestock production. Many studies have investigated the effect of growth hormone (GH), a key hormone in both growth and lactation processes in a variety of species (Bauman et al., 1986). In most studies with dairy cattle, exogenous GH has been administered in order to increase milk yield (Peel et al., 1981; Bauman et al., 1985). However, it is possible to increase endogenous GH, using compounds which regulate endogenous GH secretion. A hypothalamic growth hormone releasing factor (GRF) has been characterized (Guillemain et al., 1982) and shown to specifically induce GH secretion in various species, including cattle (Mosely et al., 1984; Hodate et al., 1985; Enrich et al., 1987). Similar to exogenous GH, administration of GRF increased milk yield in dairy cows (Enright et al., 1986, 1988; Pelletier et al., 1987).

An association between genetic merit and pancreatic response to a glucose load has been shown to exist in young animals and in lactating cows (Michel et al., 1991; Mackenzie et al; 1988). Cattle with high breeding index had significantly higher plasma INS concentrations than low breeding index cows after exogenous glucose challenge in a study conducted by Michel et al. (1988). Bovine insulin-like growth factor (IGF-I) has recently been purified and characterized. This 70 amino acid peptide is identical to human IGF-I. Insulin like growth factor is an important mediator of the biological effects of GH (Prosser et al., 1991). Growth promoting action of IGF-I has been demonstrated (Zapf et al., 1987; Schweiwiler et al., 1986) and it has been proposed to mediate the positive action of GH on milk yield in dairy cows (Bauman et al., 1986).

The objective of this study was to determine the effect of genetic selection for milk yield on the response of GH, INS and IGF-I to exogenous GRF or glucose load.

Materials and Methods

Twelve primiparous Holstein cows were housed in an open-sided free stall barn at the Virginia Tech Dairy Center and milked twice daily in a milking parlor. Animals were either daughters bred to selected, commercially available artificial insemination (AI) sires (selection group, n=6) (weighted mean sire Predicted Transmitting Ability (PTA) = 783 kg) or second to daughters of cows continuously randomly bred to non-selected sires originating in the Virginia Tech dairy herd in 1965 (control group, n=6) (Mean estimated sire Predicted Transmitting Ability (PTA= -678 kg). The estimated genetic difference in transmitting ability for the 2 groups of bulls used to sire these cows was 1461 kg milk. Daily milk yield was monitored and recorded for each cow. Phenotypic performance during the first lactation of these cattle resulted in a difference of 2360 kg of milk, with selection cows yielding 7420 kg and control cows yielding 5059 kg of milk.

Animals were fed a complete mixed ration containing corn silage, haylage, concentrate, and whole cotton seed. Whole cotton seed was added to this ration as an additional energy source. The ration averaged 55 % DM, 16 % CP and 1.56 Mcal NE/kg DM. Body weight (BW) was recorded biweekly, and converted to metabolic body weight (MBW) for use in energy balance calculations. Feed intake data was collected at 45 d intervals via an electronic intake monitoring device (Pinpointer,

Inc.) at 45 and 90 DPP. Net energy balance was calculated by the method used in study one. Data were collected through 120 DPP. The experiment was begun June, 1991 and completed February, 1992.

Serial blood samples were collected at 30, 45, 60, 75, 90, 105 and 120 DPP, via jugular cannulae inserted 3.5 h prior to sampling. Samples were collected into tubes containing 200 ul of a disodium ethylenediamine tetraacetate solution (60 mg/ml saline Na_2 EDTA, Fisher). Cows were milked at 0300 and 1500 h on the sampling day. Blood was immediately chilled on ice and then centrifuged (3,000 x g) for 20 min and plasma stored at -20 C until assayed for GH, INS or IGF-I content.

GRF Challenge

Animals were challenged with GRF (Sigma,) at 30, 60, 90, and 120 DPP. A stock solution was made by dissolving 1mg of GRF in 20 ml of deionized water (50ug/ml). Serial blood samples were collected at 15 min intervals from 0900 to 1130 h (Treatment period 1). After the 1130 h sample, GRF was administered as a bolus infusion through the jugular cannulae at a dose of .2ug/kg BW. Sampling continued at 15 min intervals until 1400 h (Treatment period 2), at which time samples were collected hourly until 2400 h (Treatment period 3).

Glucose Challenge

At 45, 75, and 105 dpp animals were challenged with a bolus infusion of glucose. A 50 % stock solution of anhydrous dextrose and deionized water was

prepared (1mg/ml). Serial blood samples were collected from 0900 to 1130 h at 15 m intervals (treatment period 1). The glucose challenge was administered at a dose of .1mg/kg BW after the 1130 h sample. Blood sampling was continued at 10 min intervals until 1230 h (Treatment period 2), and then another three samples every 10 m, finishing sampling at 15 min intervals for the last hour ending at 1400 h (Treatment period 3).

Plasma Hormone Concentrations

Plasma GH concentration was quantified using a double antibody RIA by the method of Barnes et al. (1985). The specific antibody (100 ul) used at an initial dilution of 1:1000, bound approximately 50% of labelled GH in the absence of unlabelled hormone (final volume=800 ul). Sample volumes of 300 ul plasma were assayed in duplicate and the intra- and inter-assay coefficients of variation averaged 6% and 9% respectively, in the plasma pools.

Plasma INS concentration was quantified using a double antibody RIA by the method of Barnes et al. (1985). Sample volumes of 300 ul plasma were assayed in duplicate and the intra and inter-assay coefficients of variation averaged 8% and 10% respectively, in the plasma pools.

Plasma IGF concentration was quantified using a double antibody RIA by the method of McFadden et al (in press). The specific antibody used an initial dilution of 1:9000, bound approximately 20% of labelled IGF-I in the absence of unlabelled hormone. All samples, assay volume 50 ul plasma, were assayed in duplicate and the

intra- and inter-assay coefficients of variation averaged 5% and 10% respectively, in the plasma pools. The standard ranged from 20 pg to 1.25 ng.

Data Analysis:

Hormonal data were analyzed using the General Linear Models (GLM) option of the Statistical Analysis System (SAS, 1982). Blood hormones were analyzed using a model accounting for genetic selection group, cow(genetic selection group), DPP, DPP x genetic selection group and DPP x cow(genetic selection group) interaction, period(representing pre- and post-challenge), period by genetic selection group interaction, period x DPP, period x DPP x genetic selection group, period xDPPx cow(genetic selection group). The mean square for cow within genetic selection group was used to test for differences between selection groups. The mean square for error was used to test all other effects. Metabolic body weight, dry matter intake (DMI), NEB, and ratio DMI/MY (Milk Yield) were analyzed using a reduced model. (See Appendix A, Model 2)

RESULTS

Selection cattle had greater ($P<.01$) mean milk yields (30.5 kg/d) than control cattle (23.2 kg/d) in early to mid-lactation (Figure 10). Mean milk fat yield was higher for control group cows than for selection group cows (1.65 vs 1.34 kg/d, $P<.01$) (Figure 11). Selection cattle had higher mean energy intake (EI) (23.23 vs 20.4 Mcal/d, $P<.1$) than did control group cows as shown in figure 12. Energy intake increased from 45 DPP to 90 DPP for control group cows, but remained constant for select cows.

Control and selection group cows had mean BW of 489 vs 494 kg (N.S.) during the first 12 weeks of lactation, respectively. Figure 13 depicts BW for both the control and selection group cows during early lactation. Selection cattle weighed more than controls after calving (N.S.), and lost weight until the eighth week of lactation. Selection group cattle did not regain a mean BW similar to that of mean calving weight until 16 weeks postpartum while the control group maintained their calving weight and started gaining weight at approximately 8 weeks postpartum.

Mean estimated production efficiency (conversion of feed to milk; kg milk/Mcal intake) was greater for selection group cows (1.31 vs 1.18, $P<.1$) than for control cattle (Figure 14). Production efficiency ratios were similar at 45 and 90 DPP for selection group cows, but decreased for control cows from 45 to 90 DPP. As shown in Figure 15, control group cows maintained a more positive mean NEB

Figure 10. Mean Milk Yield for Control and Selection Group Holstein Cows in Early Lactation

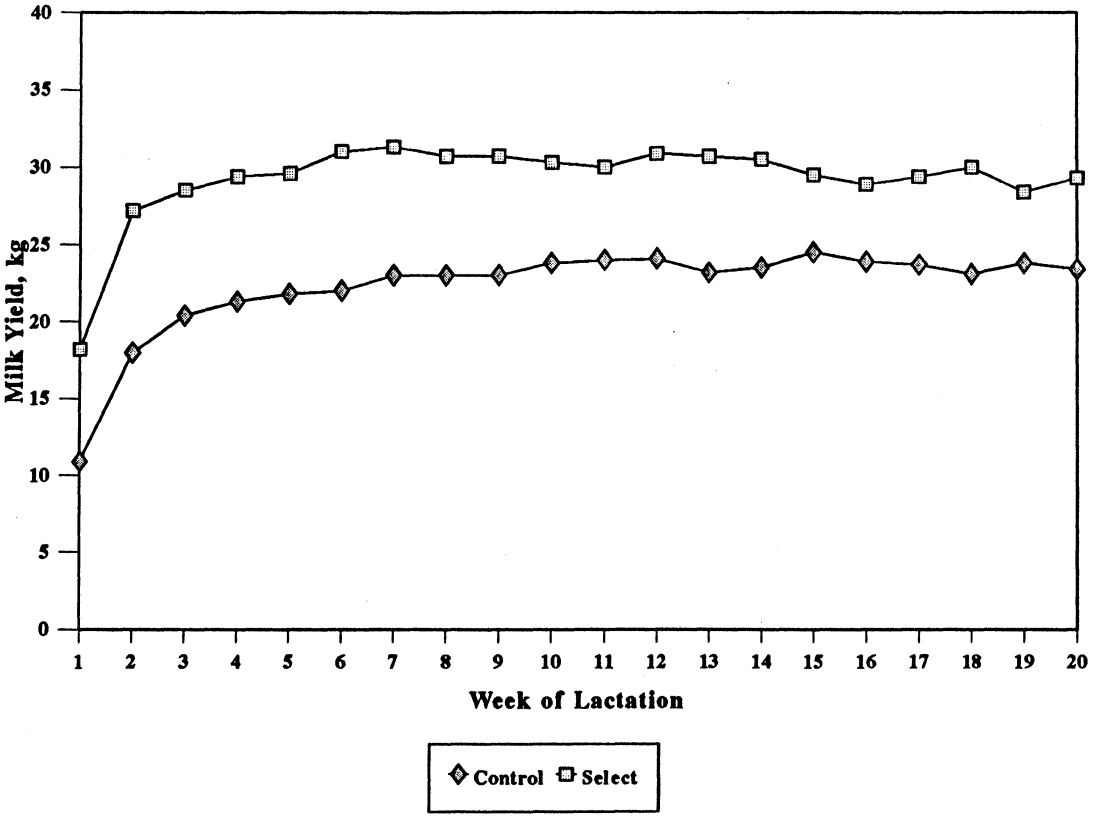


Figure 11. Mean Milk Fat Yield for Early Lactation Control and Selection Group Holstein Cows

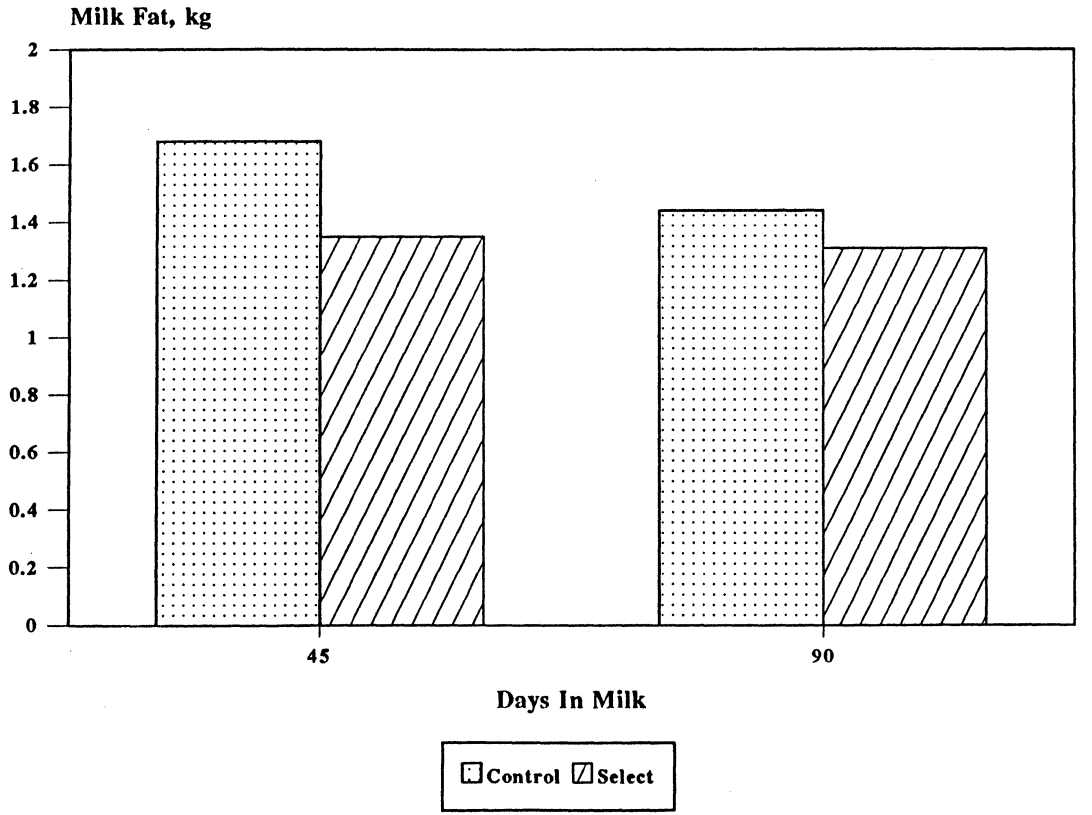


Figure 12. Mean Energy Intake for Control and Selection Group Holstein Cows in Early Lactation

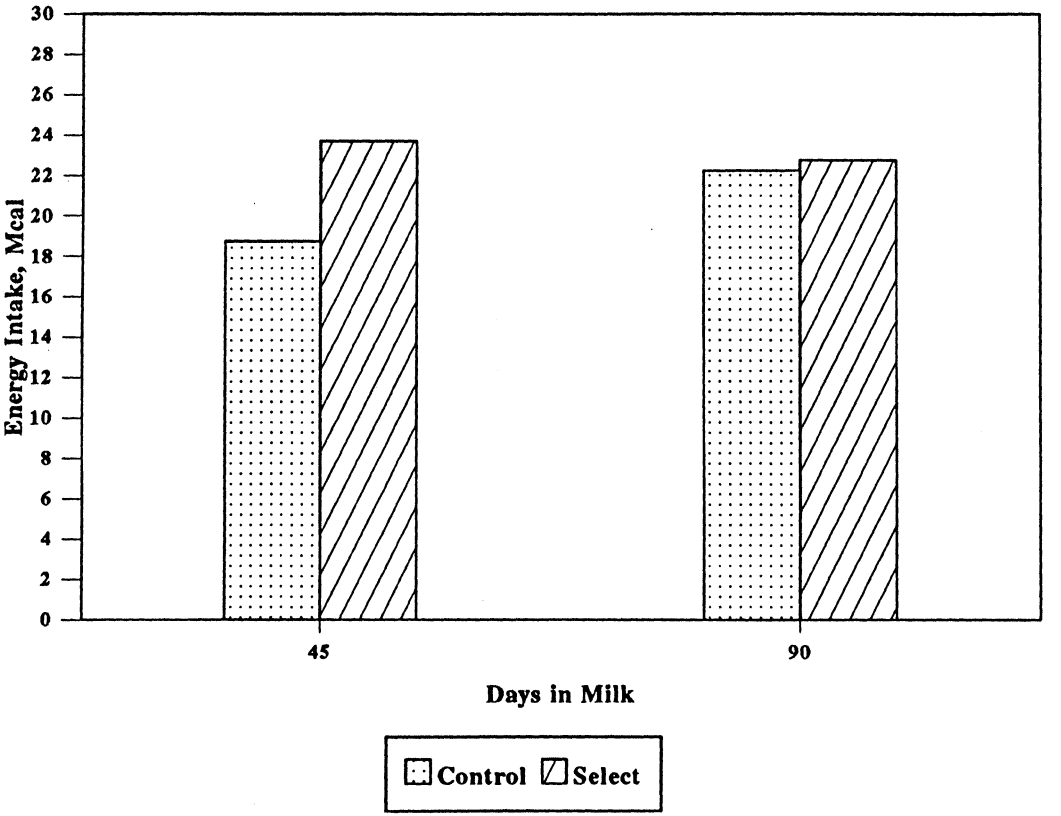


Figure 13. Mean Body Weights for Early Lactation Control and Selection Group Holstein Cows

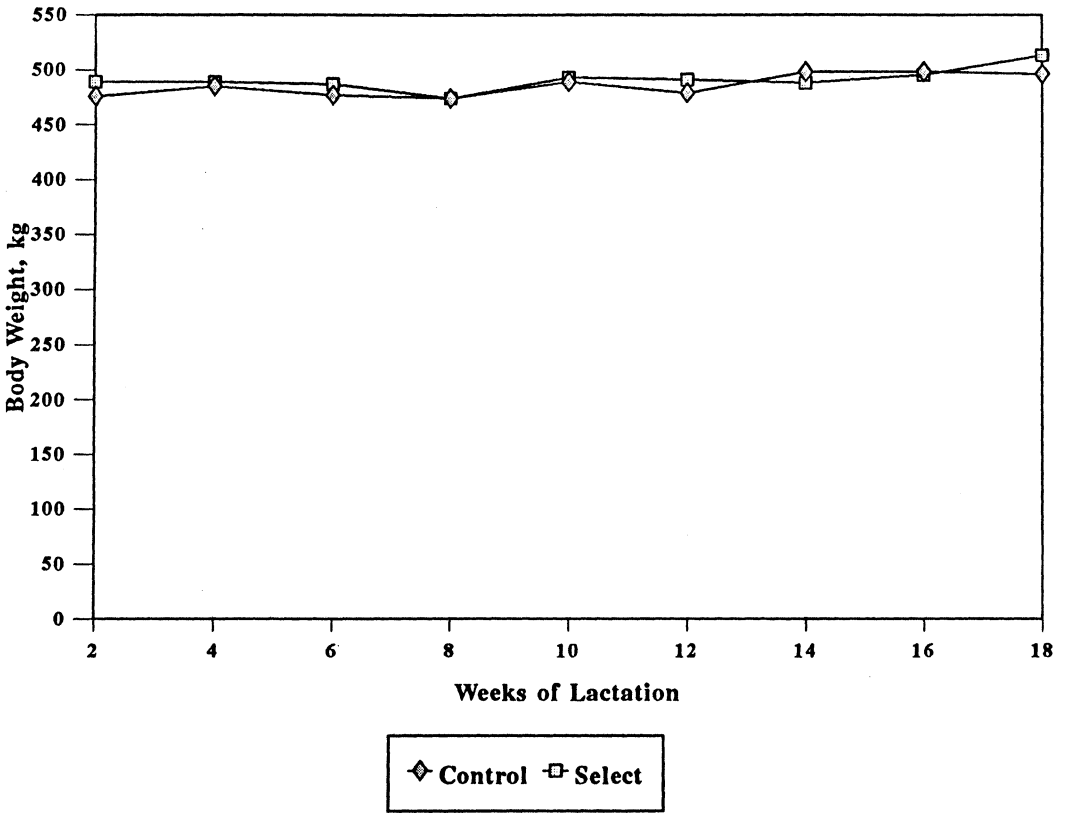


Figure 14. Mean Net Energy Balance for Control and Selection Group Holstein Cows in Early Lactation

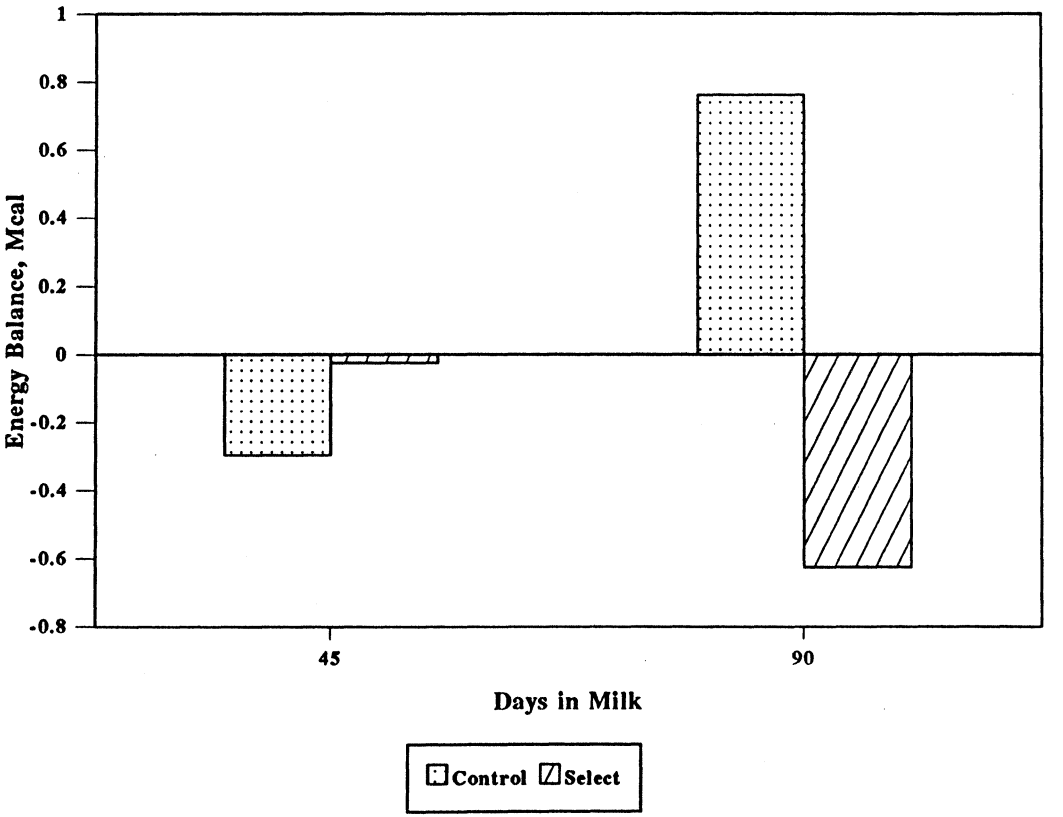
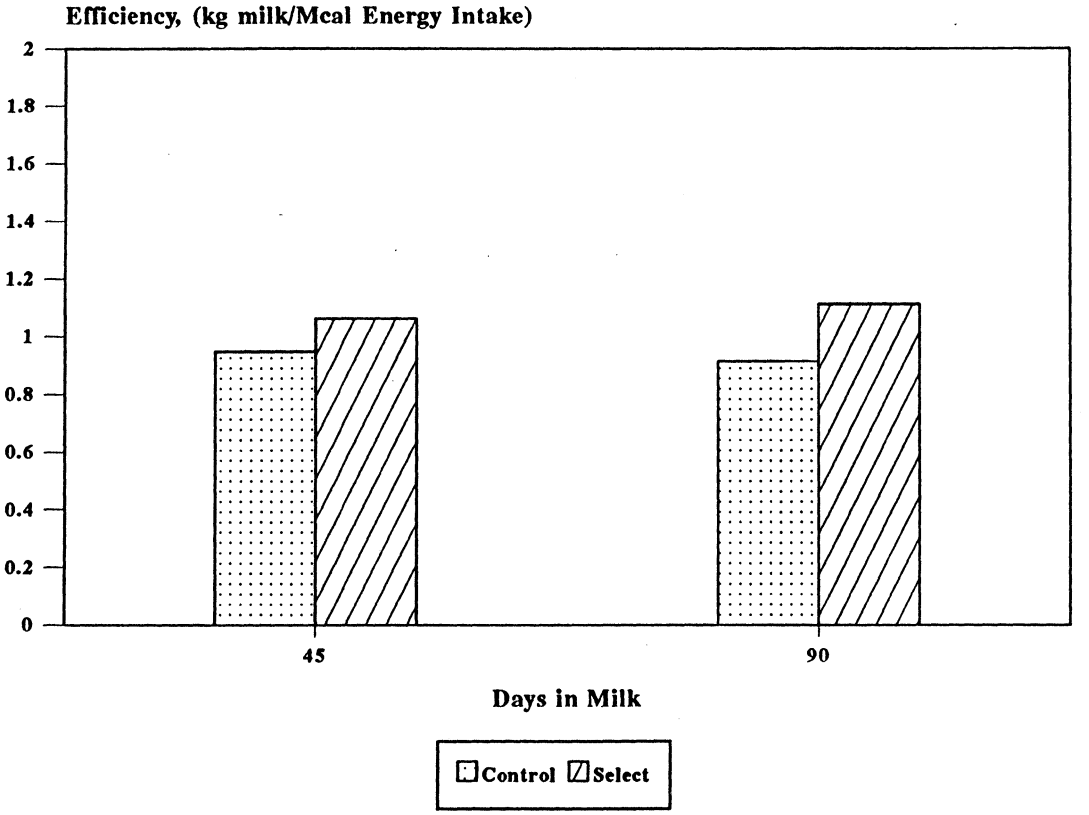


Figure 15. Mean Estimated Production Efficiency(kg milk/Mcal EI) for Control and Selection Group Holstein Cows in Early Lactation



(.23 vs .14 Mcal, P<.1) than selection cattle. From 45 to 90 DPP selection group cows declined into negative NEB, and control group cows showed an increase into positive NEB.

Mean plasma GH was higher on all test days (30, 60, 90, 120 DPP) (23.56 vs 15.6 ng/ml, P<.01) for selection compared to control group cows (Figure 16). Selection group cows showed a greater response to GRF infusion with tripling of mean basal plasma GH while plasma GH concentrations were doubled in control group cows in response to GRF challenge. Increased mean plasma GH concentration resulting from the GRF challenge returned to mean basal levels within an hour after treatment (Figures 17, 18). There was no difference in magnitude of response to challenge as lactation progressed from 30 to 120 DPP between the groups.

Mean plasma INS concentrations (Figure 19) were 1017 vs 1032 pg/ml (N.S.) for control and selection group cows, respectively. There was no difference between groups in INS response to the glucose challenge at 45, 75, and 105 DPP. Mean plasma INS concentration steadily increased in both groups, pre- and post-challenge, while on study (Figures 20, 21).

Mean plasma IGF-I concentrations tended to be greater (P<.14) in control group cows compared to selection group cows. Mean concentrations were 124 vs 98 ng/ml for control and selection cattle, respectively (Figure 22). Across all test days, 30, 60, 90, 120 DPP, and both periods (pre- and post-GRF challenge) control

cattle maintained higher ($P<.05$) plasma IGF-I concentrations (Figure 23) than selection group cows (Figure 24). No increase in plasma IGF-I concentration was observed in the thirteen hours following GRF administration in either group of cows.

Figure 16. Mean Plasma Growth Hormone (ng/ml) Concentrations in Early Lactation Control and Selection Group Holstein Cows Infused with Growth Hormone Releasing Factor (GRF, .2ug/kg BW)

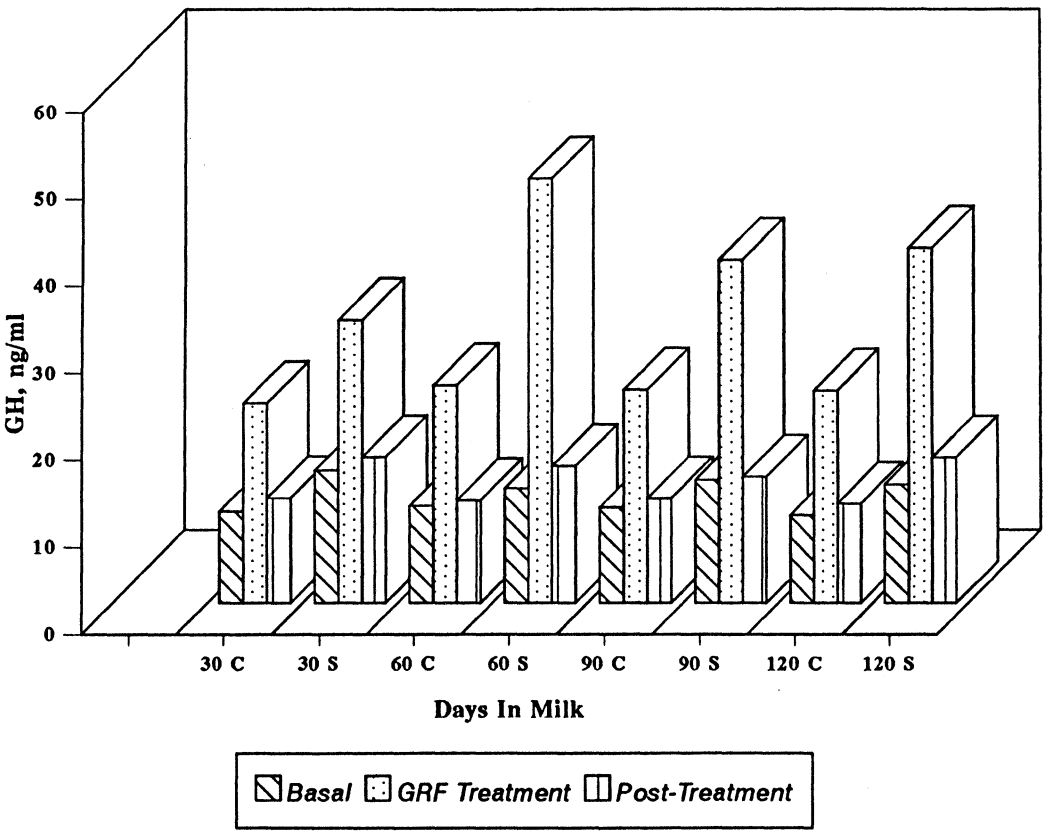


Figure 17. Mean Plasma Growth Hormone (GH) (ng/ml) Concentrations in Early Lactation Control Group Holstein Cows Treated with Growth Hormone Releasing Factor (GRF, .2ug/kg BW)

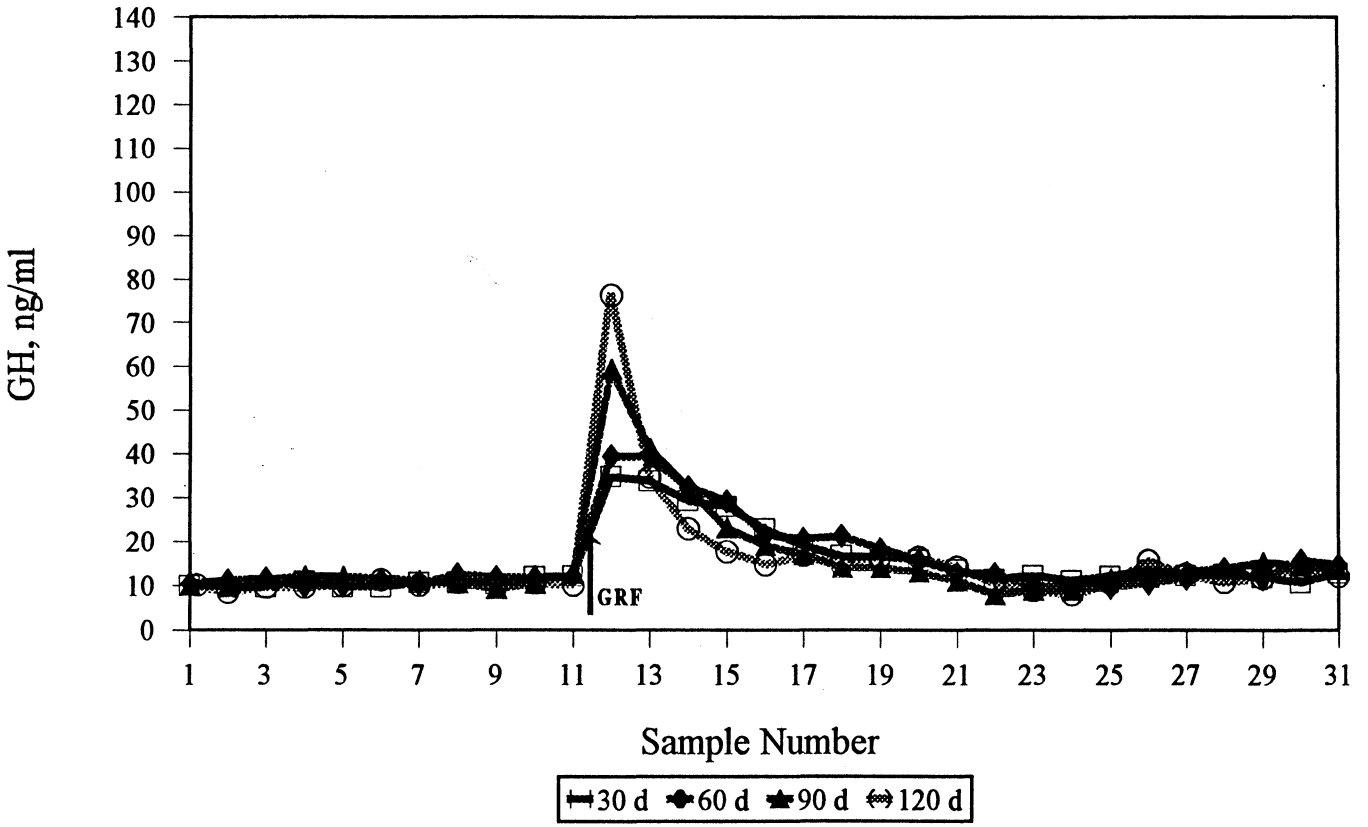


Figure 18 . Mean Plasma Growth Hormone (GH) (ng/ml) Concentrations in Early Lactation Selection Group Holstein Cows Treated with Growth Hormone Releasing Factor (GRF, .2ug/kg BW)

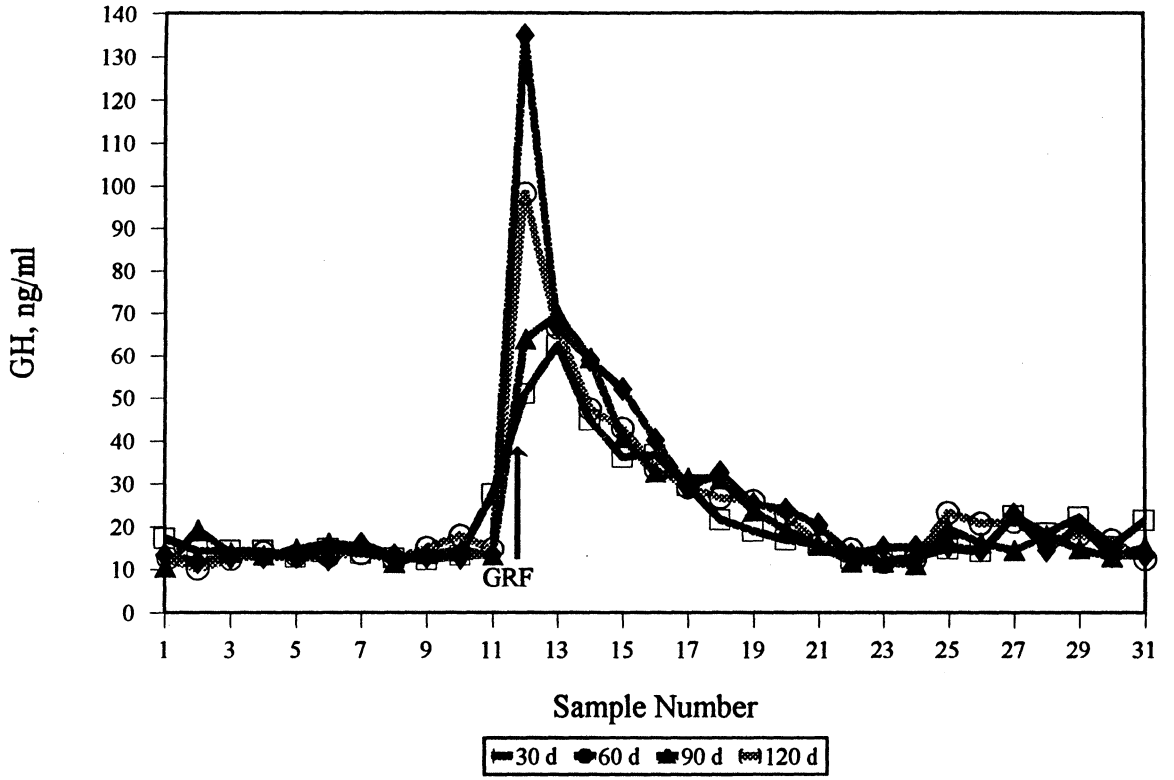


Figure 19. Mean Plasma Insulin (pg/ml) Concentrations in Early Lactation Control and Selection Group Holstein Cows Infused with Glucose (.1mg/kg BW)

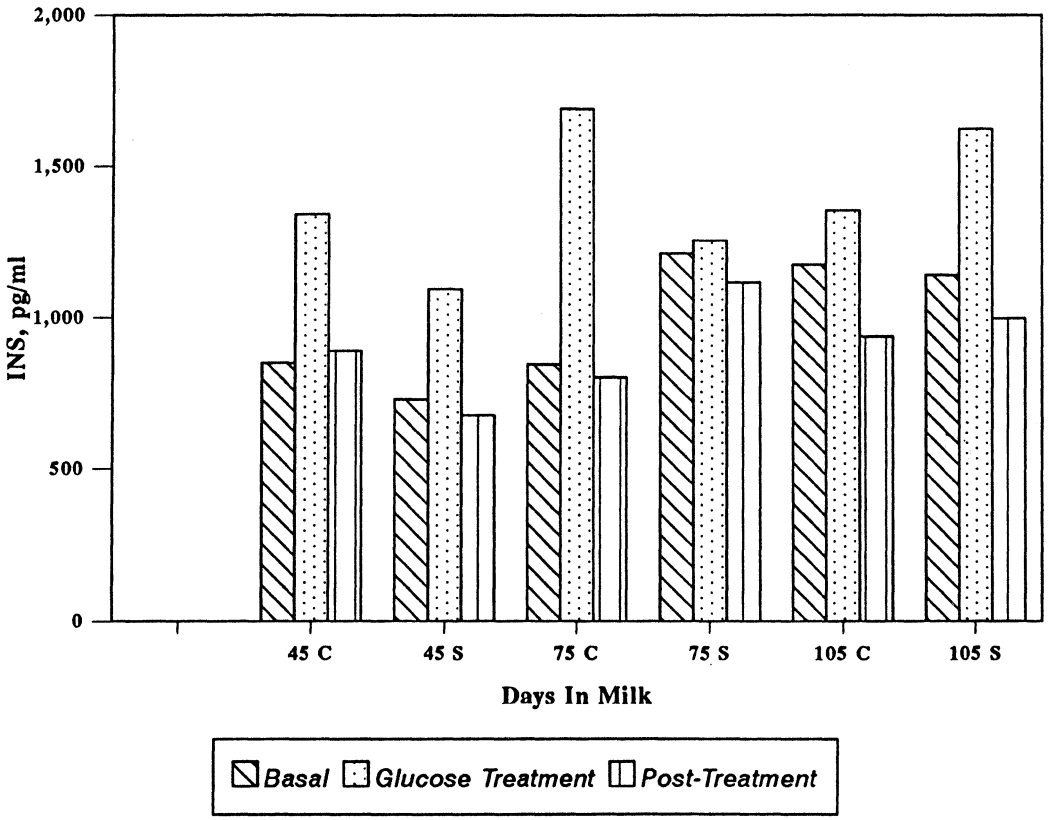


Figure 20. Mean Plasma (INS) (pg/ml) Concentrations in Early Lactation Control Group Holstein Cows Treated with Glucose (.1mg/kg BW)

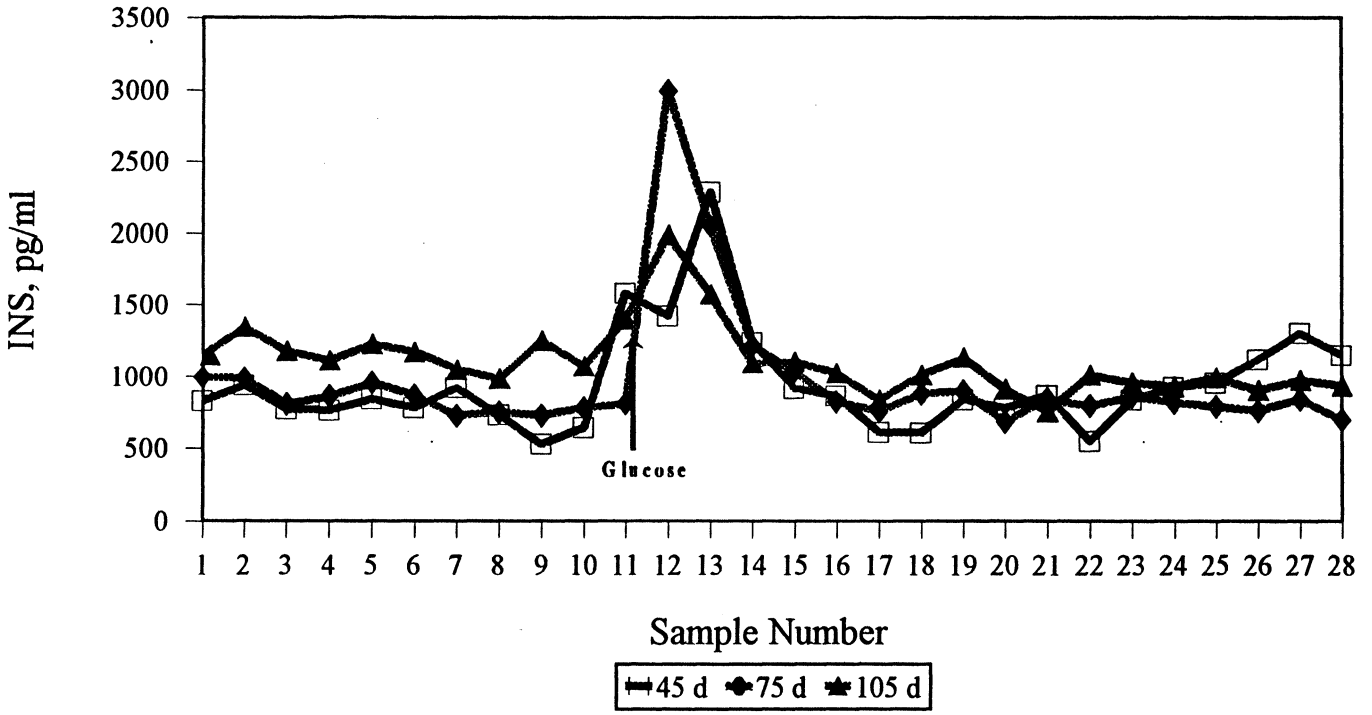


Figure 21. Mean Plasma INS (pg/ml) Concentrations in Early Lactation Selection Group Holstein Cows Treated with Glucose (.1mg/kg BW)

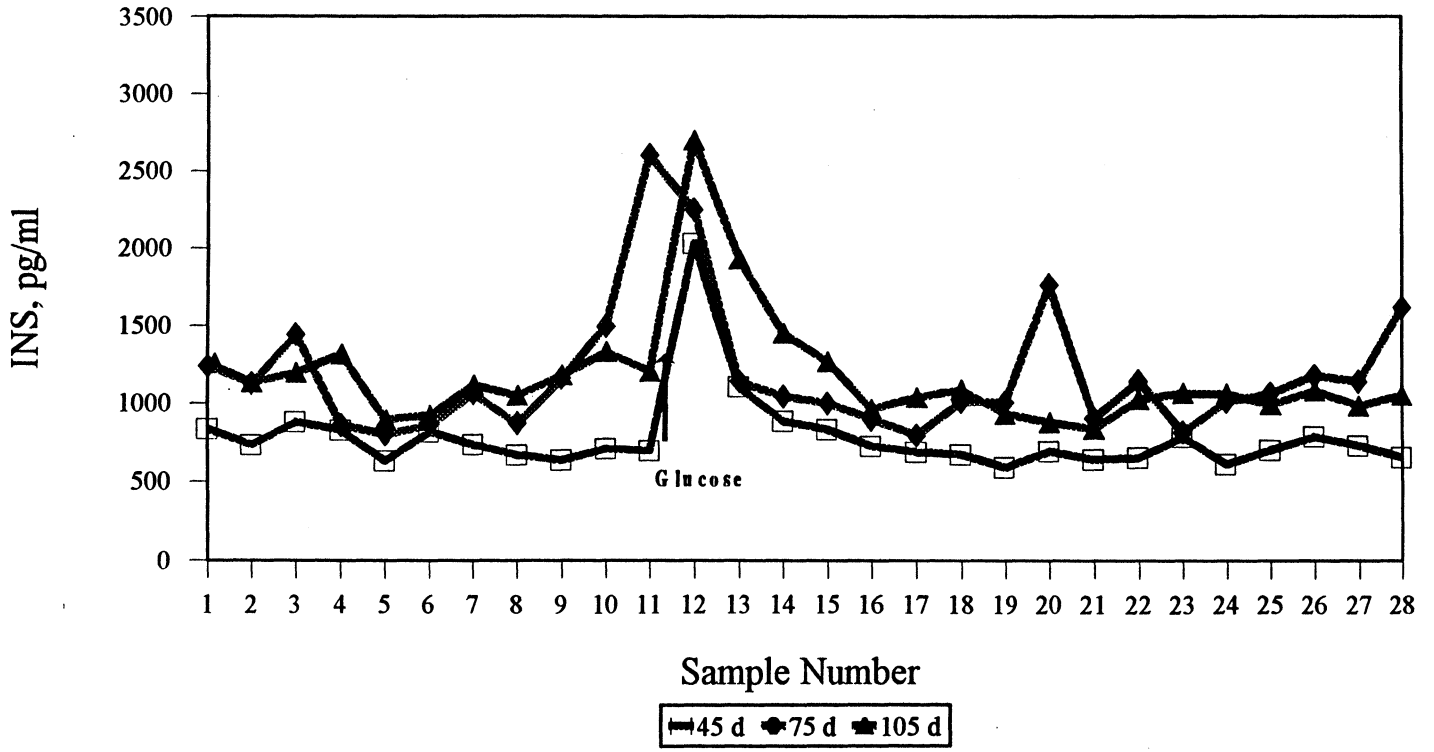


Figure 22. Mean Plasma IGF-I(ng/ml) Concentrations in Early Lactation Control and Selection Group Holstein Cows Infused with Growth Hormone Releasing Factor (GRF, .2ug/kg BW)

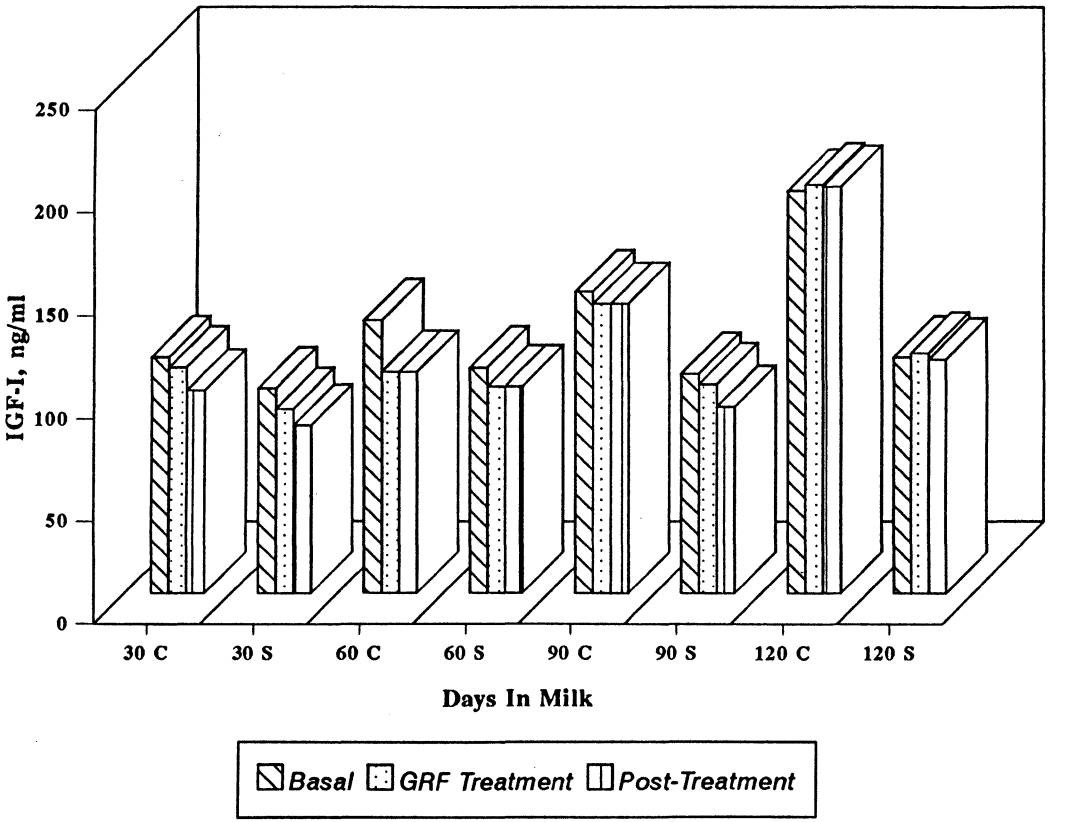


Figure 23. Mean Plasma Insulin Like Growth Factor (IGF-I) Concentrations in Early Lactation Control Group Holstein Cows Treated with Growth Hormone Releasing Factor (GRF, .2ug/kg BW)

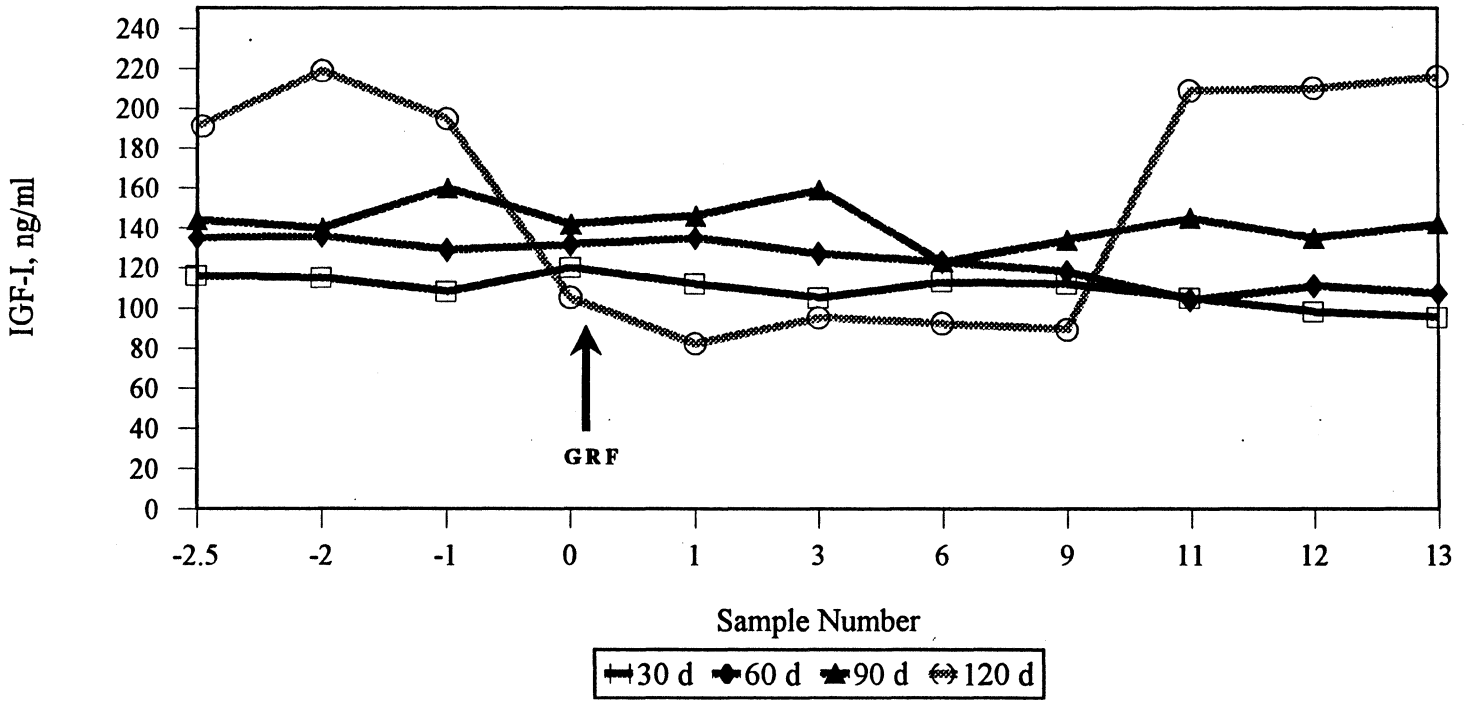
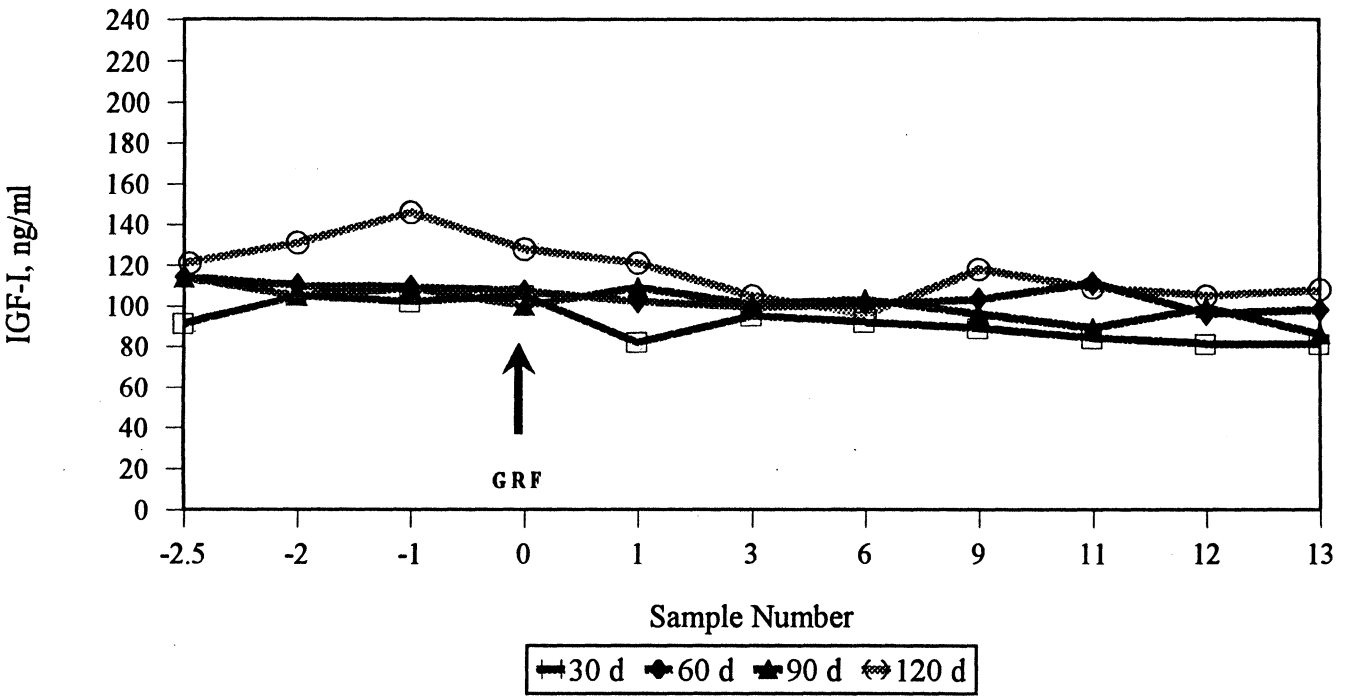


Figure 24. Mean Plasma Insulin Like Growth Factor (IGF-I) Concentrations in Early Lactation Selection Group Holstein Cows Treated with Growth Hormone Releasing Factor (GRF, .2ug/kg BW)



DISCUSSION

Energy intake was greater ($P<.1$) in selection cows as compared to control group cows, similar to findings of Barnes et al. (1985) and Kazmer et al. (1986) where DMI was significantly greater in first lactation daughters of A.I. sires, compared to lower producing control cows. Selection cows were characterized in the present study as more efficient producers at 45 and 90 DPP because selection group cows had a higher ratio of feed to milk conversion compared to control cows in early lactation. Bryant and Trigg (1981) reported that cows of higher genetic merit for milk yield were more efficient producers in terms of utilizing feed intake for milk production compared to cows of lesser genetic merit for milk yield.

Hart et al. (1979) investigated differences in endocrine control of energy metabolism between high-yielding Holsteins, and low-yielding HolsteinxHerefored crossbreds. In that study, higher producing cattle lost weight to week 14 of lactation and did not regain original calving weight until week 30 of lactation, while the lower producing cattle did not lose weight in early lactation but gained weight continually throughout lactation. In Hart's experiment, high- and low-yielding cows were fed the same quantity of an identical ration. Therefore, the higher-yielding Holstein cows were being underfed with respect to energy needs for production and rapidly lost weight while lower-yielding control cows were being overfed with respect to production needs and gained weight. However, when high- and low-yielding dairy

cattle are fed ad libitum, as in the present study, and in other studies (Bines et al. 1983; Barnes et al., 1985; Kazmer et al., 1986) the patten of weight loss in early lactation and gradual increase in BW as lactation progresses with declining demand for energy for milk synthesis is well documented. These studies have also demonstrated that control group cattle regain calving weight more quickly than selection group cows during lactation.

Selection cows were in a more negative NEB at 45 and 90 DPP compared to control cows. Selection group cattle yielded almost 10 kg/d more milk than control cattle, through 135 DIM, thus creating a greater energy deficit in selection than in control cows. The fact that selection cattle regained their original calving weight more slowly during early lactation compared to control cows was also a result of their being in a more negative NEB.

Although the calculated value for NEB is only an estimate of energy inputs and losses, it provides a necessary framework of reference for comparing energy status between groups of differing genetic merit. Both Moe (1981) and Bauman et al. (1980) reported genetically superior dairy cows tended to prioritize nutrients to be used for milk synthesis and therefore were in a more negative NEB than lower-yielding cows, especially in early lactation. Similarly, Lukes et al. (1989) reported selection and control cows were in negative NEB at parturition and 45 DPP, a period of weight loss and increasing demand to synthesize milk. In contrast to these findings Kazmer et al. (1986) reported that first and second lactation selection and control

group cows, fed a total mixed ration ad libitum, had a similar calculated NEB throughout lactation. Kazmer et al. (1986) reported negative NEB at 30 DPP and a positive NEB at 90 and 200 DPP in both selection and control cows.

In the current study selection cows were in negative NEB at 45 and 90 DPP and control cows were in negative NEB at 45 DPP and increased to a slightly positive NEB by 90 DPP, corresponding to the period of post-calving weight loss and increased milk yield. However, selection cows were in a more negative NEB than control group cows as reflected by their greater weight loss and elevated milk yield compared to control cows during early lactation.

Of the hormones measured in the present study, the most consistent difference between genetic groups was in GH concentration. These findings are in agreement with those reported by Lukes et al. (1989). Plasma GH concentration was greater overall in selection compared to control group cows. Kazmer et al. (1986) reported that dairy cows selected on genetic potential for milk yield had greater plasma GH concentrations than control group cows before and after exogenous thyrotropin releasing hormone administration. Similar to the study of Lukes et al. (1989), mean basal GH concentrations of selection group animals was greater at 30, 60 , 90, and 120 DPP compared to control cattle.

In the current study, GRF stimulated a GH response in both selection and control groups, at every test day during the trial. However, the magnitude of the response varied between groups; with greater GRF induced GH response occurring

in the selection group cows compared to control group cows. Sartin et al. (1985) reported that the GH response to GRF challenge increases in magnitude with advancing lactation due to decreasing concentrations of somatostatin, a small peptide secreted by the CNS which inhibits GH release (Berelowitz et al., 1981). In the present study plasma GH concentrations increased progressively on days 30, 60, 90, 120 of lactation. This is also in agreement with a report by Lukes et al. (1989).

Enright et al. (1986) took continuous samples after administering GRF in Holstein steers and reported peak GH concentration within approximately 20 min post-injection. Similarly, in the present study, peak plasma GH was measured 15 min post-injection. However, it is possible that peak GH response occurred before or after the 15 min post-injection sample, and additional, more frequent sampling would be required to ascertain actual peak hormone concentration. The increased GH response to exogenous GRF in selection cows at 30, 60, 90, and 120 DPP compared to control cows may be due to an increased intrinsic sensitivity of the anterior pituitary to GRF compared to control group cows or possibly greater anterior pituitary GH stores in selection cows. Alternatively, selection cows may have decreased somatostatin concentrations or be unaffected in early to mid lactation by the inhibitory effect of somatostatin.

In the present study, basal plasma INS concentrations increased gradually as lactation progressed; corresponding to a shift in metabolism from a catabolic to an anabolic state as lactation progresses and energy demand for milk yield decreases

(Kazmer et al., 1991). In the ruminant, INS promotes an anabolic state in its effects on carbohydrates, lipid, and amino acid metabolism (Bassett, 1975). Insulin stimulates glycogen, FFA and triglyceride synthesis as well as protein synthesis (Basset, 1975). There was no significant INS response to the glucose infusion by either group at 45, 75 or 105 DPP. Michel et al. (1991) found high breed index cows had significantly higher plasma INS concentration than low breed index cows after a glucose challenge, irrespective of whether they had received bST treatment or control formulation. Corresponding effects on basal INS concentration were not observed in the present study, possibly due to a depressing effect of negative NEB while being challenged. Energy balance can affect plasma concentrations of hormones responsible for nutrient partitioning. Cows in greater negative NEB tend to have depressed concentrations of INS (Cummins et al., 1987).

Mean plasma IGF-I concentrations were not altered by infusion of GRF. Mean concentrations of IGF-I tended to be greater on all test days, 30, 60, 90 and 120 DPP in the control group cows compared to selection cattle. Binners et al. reported that high GH concentration during peak milk yield was not related to high blood IGF-I concentration. Animals in a more positive NEB tend to have higher plasma IGF-I concentrations (Spicer et al., 1990), which corresponds to the nutritional state of the control group cows in the present study. In contrast the selection group cows were in negative NEB and displayed lower plasma IGF-I concentrations. Arbitat et al. (1990) found IGF-I concentration in Holstein cattle

increased following either an acute GRF injection or GH injection, thus indicating a GH effect on IGF-I concentration regardless of whether GH concentration increased through exogenous administration of GH or GRF. The IGF-I response to GRF was delayed compared to the response of GH and was maximal 8 to 10 hour after GRF injection. Lapierre et al. (1990) conducted a study with thirty cows averaging 196 d of lactation. Cattle in the treatment group received GRF for 10 consecutive days at a dose of 10 ug/kg BW. The authors reported that GRF treatment increased IGF-I concentrations. In a follow up study by Lapierre et al. (1990), a GRF analog administered to dairy cows at .6 ug/kg BW was used to obtain a similar GH and IGF-I response to that of the 10 ug/kg BW dose of hGRF. Since similar results were not found in the present study, the most likely reason can be dose attributed. Arbitat et al. (1990) administered GRF at a dose of 10ug/kg BW, whereas in the present study the GRF dose used was .2ug/kg BW. The dose used in the present study has been reported to be sufficient to cause increased GH release and GH was increased in response to this dose in the present study.

CONCLUSIONS

Results indicated that selection pressure for milk yield in first lactation Holstein cows resulted in more efficient production(kg milk/kg EI) at 45 and 90 DPP, increased milk yield, decreased NEB, greater postpartum weight loss, and elevated concentration of plasma GH. Consistent effects of selection for milk yield on plasma INS and IGF-I concentrations were not evident, indicating no apparent direct effects of selection pressure on regulation of these hormones. Increased milk yield efficiency in selection cows may result from the interactions of GH with these hormones or other growth factors.

The fact that IGF-I response to GRF challenge did not occur in this study may be a result of depressed energy balance, but possibly the super physiological doses of GRF used in other studies to increase IGF-I led to results not seen in this study. In any case, it appears that plasma IGF-I concentrations are not a strong indicator of potential milk yield ability for dairy cows since, while the low-yielding animals continually displayed higher concentrations of IGF-I compared to selection group cows in this study, no strong statistical difference in IGF-I between selection groups was detected.

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APPENDIX A

Model 1:

$$Y = u + G_j + C_{ij} + D^k + GD_{ik} + GCD_{ijk} + E_{ijkl}$$

Where:

Y = GH, INS, IGF-I, BW, Energy Intake, Energy Balance, Milk and Milk fat

u = population mean

$\mathbf{G_i}$ = i^{th} group

$\mathbf{C_{ij}}$ = j^{th} cow in the i^{th} group

$\mathbf{D_k}$ = k^{th} days in milk

$\mathbf{GD_{ik}}$ = interaction of i^{th} group and k^{th} days in milk

$\mathbf{GCD_{ijk}}$ = interaction of j^{th} cow in the i^{th} group with k^{th} days in milk

$\mathbf{E_{ijkl}}$ = residual error

STATISTICAL ANALYSIS

Model 2:

$$Y = u + G_i + C_{ij} + D_k + GD_{ik} + GCD_{i(j)k} + P_l + PG_{il} + PC_{ijl} + PD_{ikl} + PGD + PCD + E_{ijkl}$$

Where:

Y = GH, INS, IGF-I, BW, Energy intake, Energy balance, Milk and Milk fat

u = population mean

$\mathbf{G_i}$ = i^{th} group

$\mathbf{C_{ij}}$ = j^{th} cow in the i^{th} group

$\mathbf{D_k}$ = k^{th} days in milk

$\mathbf{GD_{ik}}$ = interaction of i^{th} group and k^{th} days in milk

$\mathbf{GCD_{i(j)k}}$ = interaction of j^{th} cow in the i^{th} group with k^{th} days in milk

$\mathbf{P_l}$ = effect of l^{th} period

$\mathbf{PG_{il}}$ = interaction of the i^{th} group and l^{th} period

$\mathbf{PC_{ijl}}$ = interaction of j^{th} cow in the i^{th} group with k^{th} days in milk

$\mathbf{PD_{ikl}}$ =interaction of the k^{th} days in milk with the i^{th} group in the l^{th} period

$\mathbf{PCD_{ijkl}}$ =interaction j^{th} cow and k^{th} days in milk and the ith group with the l^{th} period

$\mathbf{E_{ijklm}}$ = residual error

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